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SELECTED ESSAYS

ON

SYPHILIS AND SMALL-POX.

TRANSLATIONS AND REPRINTS FROM
VARIOUS SOURCES.

EDITED BY

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WITH ILLUSTRATIONS AND CHARTS.

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PREFACE.

In the selection of the following essays for publication the Council of the New Sydenham Society has been guided by a desire to present papers illustrating the progress of current experimental research. It has been felt that the experiments and observations on syphilis and smallpox here recorded are of such extreme importance that the dissemination of the original papers themselves will be widely welcomed.

Much of the work under discussion is obviously of permanent and extreme value. It is, however, being subjected to the most severe scrutiny by observers all over the world, and it may be that some of the conclusions now arrived at will require to be modified in the light of the wider knowledge which future work will establish.

CONTENTS.

PAGE

THE OCCURRENCE OF SPIROCHÆTES IN SYPHILIS, BY DR. FRITZ SCHAUDINN AND DR. ERICH HOFFMANN 1-59

A Preliminary Note upon the Occurrence of Spirochætes in Syphilitic Lesions and in Papillomata, by Drs. Schaudinn and Hoffmann 3-15

Methods of Preparation and of Staining 4

Description of the Organism 5

Presence of Spirochætes in Cases of Uncomplicated Syphilis 7-10

Presence of Spirochætes in Cases of Syphilis Complicated by other Infections 10-12

Presence of Spirochætes in Non-Syphilitic Diseases ... 12-13

The Occurrence of Spirochætes in the Fluid Obtained from the Lymphatic Glands in Syphilis, by Drs. Schaudinn and Hoffmann 16-25

Method of Obtaining the Lymphatic Fluid 17

Description of the Organism 18-19

Cases 20-24

The Occurrence of Spirochæta Pallida in Syphilis and the Differences Between this Form and Others of the Same Family, by Drs. Schaudinn and Hoffmann 26-35

Further Remarks upon the Occurrence of Spirochæta Pallida in Syphilis, by Dr. Hoffmann 36-43

Confirmation by other Observers 37

Distribution of the Parasite in the Tissues 39

Presence in Congenital Syphilis 41

PAGE
THE SPIROCHÆTA PALLIDA, BY DR. SCHAUDINN 44-51
  Biological Relationships of Spirochæta Pallida 45
  Characteristics of Spirochæta Pallida 46-48
  Presence of Flagella 49-50
  Nomenclature 51
SPIROCHÆTA PALLIDA IN A MACAQUE INOCULATED WITH BLOOD FROM A CASE OF SYPHILIS, BY DR. HOFFMANN 52-54
FURTHER COMMUNICATION UPON SPIROCHÆTA PALLIDA, BY DR. HOFFMANN 55-59
  Position of the Spirochætes in the Tissues 57
  Diagnostic Significance of the Spirochætes 58
SPIROCHÆTA PALLIDA IN SYPHILIS, ABSTRACT OF PAPER BY DR. THEODORE SHENNAN 60-79
  Résumé of Literature 60-73
  Description of the Parasite 73-74
  Conclusions 76-77
  References 77-79
THE PRESENCE OF SPIROCHÆTES IN YAWS, ABSTRACT OF PAPERS BY DR. ALDO CASTELLANI 80-83

EXPERIMENTAL INVESTIGATIONS ON SYPHILIS, BY PROFESSORS EL. METCHNIKOFF AND EM. ROUX 85-135

FIRST COMMUNICATION 87-95
  Early Experiments 87-90
  Inoculation of a Chimpanzee 91
SECOND COMMUNICATION 96-101
  Inoculation of Macaque Monkeys 97
  Inoculation of Chimpanzee from Macaque 98
THIRD COMMUNICATION 102-114
  Incubation Period in the Chimpanzee... 103
  Secondary Symptoms in the Chimpanzee 105

PAGE
Properties of Syphilitic Virus when Filtered... 106
Properties of Syphilitic Virus after Treatment by Heat and Glycerine 107-108
Negative Vaccine Experiments... 109
Syphilis in the Lower Catarrhine Apes 111-113
Fourth Communication 115-135
Syphilis in Anthropoid Apes, General Symptoms 115-121
Attempts at Preventive Treatment 121
Anti-syphilitic Sera 122-126
Negative Vaccine Experiments 126-127
Mercurial Inunction 128-130
The Micro-organism of Syphilis 130
Summary of Inoculation Experiments 133-135

STUDIES ON THE PATHOLOGY AND ON THE ETIOLOGY OF VARIOLA AND VACCINIA 137-215
The Life History of Cytoryctes Variolæ Guarnieri, by Prof. Gary N. Calkins 139-172
Methods of Staining 146-147
Phases of the Life Cycle of the Parasite 148-161

(1) *The Early Cytoplasmic Stages.*

(a) The Youngest Forms 149
(b) Growth and Differentiation... 150
(c) The Adult Amœboid Organism and Gemmule Formation 151
(d) The Cytoplasmic Residuum 152

(2) *The Early Intra-nuclear Phase.*

(a) The Gametocytes 153-156
(b) The Zygote 156
(c) The Pansporoblast 157

(3) *Secondary Nuclear Phases.*

(a) The Spore 159
(b) The Secondary Sporoblast and its Sporulation 160
(c) The Sporoblast Residuum 161

PAGE

Biological Considerations.

(1) Multiplicative Reproduction 161-166
(2) The Distributed Nucleus 166
(3) Caryoryctes Cytoryctoides 168-169
Scheme of Classification 170-171
Bibliography 172

EPICRISIS, BY PROF. W. T. COUNCILMAN 173-189

CLINICAL OBSERVATIONS ON VARIOLA, BY DR. I. R. BANCROFT 191-215
Variola Vera 193
Initial Stage 194
Stage of Eruption 197
Temperature 202
Complications 204
Abortive Types 206
Variola Sine Eruptione 207
Variola Pustulosa Hæmorrhagica 208
Purpura Variolosa 209
Initial Rashes 212
Secondary Rashes 214

ILLUSTRATIONS.

Microphotographs of Spirochæta Pallida 6
Microphotograph of S. Pallida and S. Refringens 29
Varieties of Spirochætæ 50
Sporoblast Residuum of Thèlohania Mülleri 161
Life cycle of Cytoryctes Variolæ 162
Gemmule Formation in Bacillus Bütschlii 163
Malaria Parasite 168
Microsporidia with Vacuolated Spores 170

CHARTS.

Chart 1. Age Incidence of Variola 194
,, 2. Variola Vera, Typical Case 200
,, 3. Variola Vera, Severe Case 201
,, 4. Variola Vera, Severe, with Crisis on Fourteenth Day 202
,, 5. Variola Vera, Fatal on Fourteenth Day 203
,, 6. Variola Pustulosa Hæmorrhagica 209
,, 7. Variola Vera, with Secondary Rash 215

ESSAYS UPON

The Occurrence of Spirochætes in Syphilis

BY

Dr. FRITZ SCHAUDINN

AND

Dr. ERICH HOFFMANN.

TRANSLATED BY T. G. BRODIE, M.D., F.R.S.

A PRELIMINARY NOTE UPON THE OCCURRENCE OF SPIROCHÆTES IN SYPHILITIC LESIONS AND IN PAPILLOMATA.

BY

FRITZ SCHAUDINN and ERICH HOFFMANN.

(From the *Arbeiten aus dem Kaiserlichen Gesundheitsamts*,
Bd. 22, Heft 2, April 10, 1905).

On the suggestion of Professor Köhler, President of the Imperial Board of Health, and under the assistance of Professor E. Lesser, some investigations upon the occurrence of micro-organisms in syphilitic lesions have been commenced by us in association with Drs. Neufeld and Gonder. In the course of these, Schaudinn found in the living subject as well as in stained preparations, organisms which must be referred to the species *Spirochæta*, a species whose systematic position in the family of the Protozoa, Schaudinn has made probable from his investigations upon the *Spirochæta ziemanni* of the owl. Up to the present, *the spirochætes have been found both on the surface of secreting syphilitic eruptions, as well as in the deeper parts of the tissues and in specifically infected inguinal glands.*

In order to allow of the early confirmation of this discovery, the results, illustrated by two microphotographs, are now published in a brief form.

In addition to pure cases of syphilis, further cases complicated by other diseases were also investigated. Finally, attempts to discover similar organisms in the genital regions of the body in patients not suffering from syphilis have also been made.

In the following pages we give, first a brief description of the methods employed and the characters of the parasites discovered, and secondly, a short sketch of the cases examined with the results obtained in the examination for the parasite.[1]

Freshly-collected material is best fitted for the finding of the extraordinarily delicate, weakly refracting, but very actively motile spirochætes in the syphilitic tissues. This is examined immediately after removal from the body by the ordinary cover-glass preparations. In the case of the primary lesions, large papules and condylomata were chosen and a drop of the tissue juice, expressed as nearly as possible from the middle of the tissue, was collected on a cover-glass and at once examined. In the case of the glands, which were extirpated *in toto*, these were rapidly cut across and a drop of fluid taken from the centre.

To demonstrate the spirochætes in stained preparations a thin cover-glass film from the same parts of the infected tissue was taken, and after it had dried in the air, was fixed for about ten minutes in absolute alcohol. The spirochætes behave differently with regard to staining properties in the various cases examined. Two forms can be distinguished, the first of which is characterised, in the living state, by its somewhat stronger refrangibility and slightly coarser form, as well as by appearing to possess wide and flat turns in the spiral. It could be easily picked out by staining with the ordinary reagents employed for spirochætes (gentian-violet, carbol-fuchsin, Romanowsky stain, &c.). In the list of cases this variety is termed "the darkly staining type." At present they have not been found in uncomplicated syphilitic lesions, but have always been demonstrated in gonorrhœal warts (five cases). The second group consists of spirochætes, which, while living, are extremely delicate, weakly refractile, and possess steep and narrow spirals. It is not easy to obtain preparations of them by the ordinary staining methods. At present, it has only been possible to bring out the spirochætes clearly in preparations by means of the following modification of Giemsa's azur-eosin stain. The well-fixed films were placed for sixteen to twenty-four hours in a freshly-prepared mixture of: (1) 12 parts Giemsa's eosin solution (2·5 cc. 1 per cent. eosin solution in 500 cc. water); (2) 3 parts azur I. (1 in 1,000 of water); (3) 3 parts azur II. (0·8 in 1,000 of water).

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[1] The details described, so far as they refer to the parasitology, are given by Schaudinn, those dealing with the clinical and literary part by Hoffmann. We are greatly indebted to Dr. Gonder for much help in the preparation, staining and examination of the preparations.

After washing for a short time in water the cover-glass is dried and examined in cedar oil. Such preparations do not show this second type of spirochæte by any means so clearly as the first, but yet do so sufficiently well to enable them to be photographed (see the two figures in the text). This form is termed the "pale type" in the list of cases. It alone is found in all the uncomplicated syphilitic lesions.

Other definite distinctions between the two forms have not yet been discovered. The study of the developmental history of this parasite can alone teach us whether we are here dealing with two distinct species.[1] The following description relates to the "pale form."

The length of the spirochæte varies from 4 to 10 μ, the average being about 7 μ, as a comparison of the spirochætes of fig. 1 with the red blood corpuscle seen in the centre of the photograph will show. Hence our type may be smaller than most species of spirochæte (*S. obermeieri*, *anserina*, *ziemanni*, *buccalis*). The width varies from a non-measurable size to about 0·5 μ in the largest forms of the deeply-staining variety. The number of turns of the spiral fluctuates between three and twelve. For the species spirochæte as compared to spirillum, the movements during life are characteristically of three kinds: rotation about the long axis, gliding movements forwards and backwards, and movements of flexion of the whole body. Indications of an undulating membrane are at times to be perceived, but there is no evidence of the presence of flagella. The ends

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[1] Should the conjecture that we are dealing with two distinct forms be confirmed, I propose the name *S. pallida* for the "pale form," and *S. refringens* for the deeply-staining one.—SCHAUDINN.

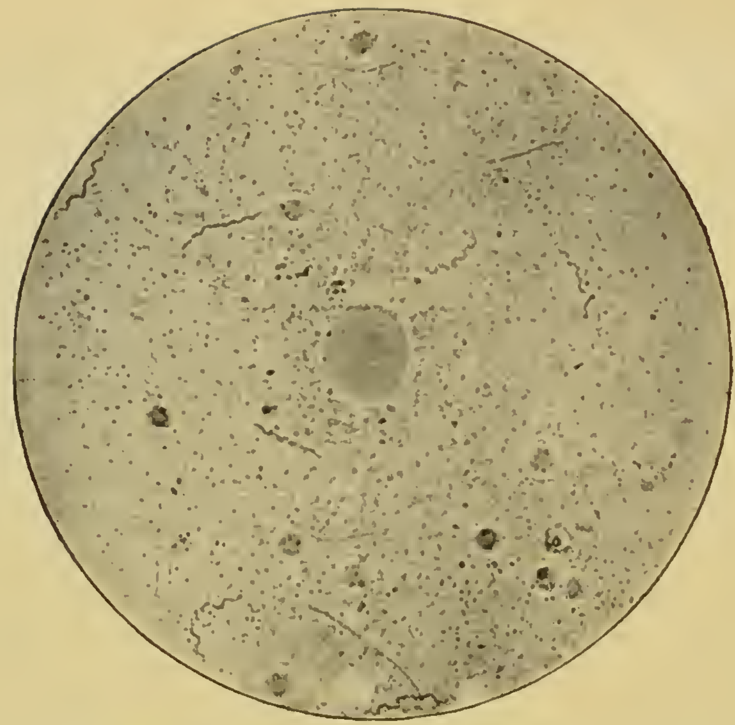

FIG. 1.—Photograph of a film from a specific papule (Giemsa stain). There is a red blood corpuscle in the centre of the field, and seven spirochætes are clearly seen in the surrounding material. Mag. ca. 1200.

(*Cf.* Case 4 of the List.)

(Prepared by Gonder. Photogr. by Schaudinn.)

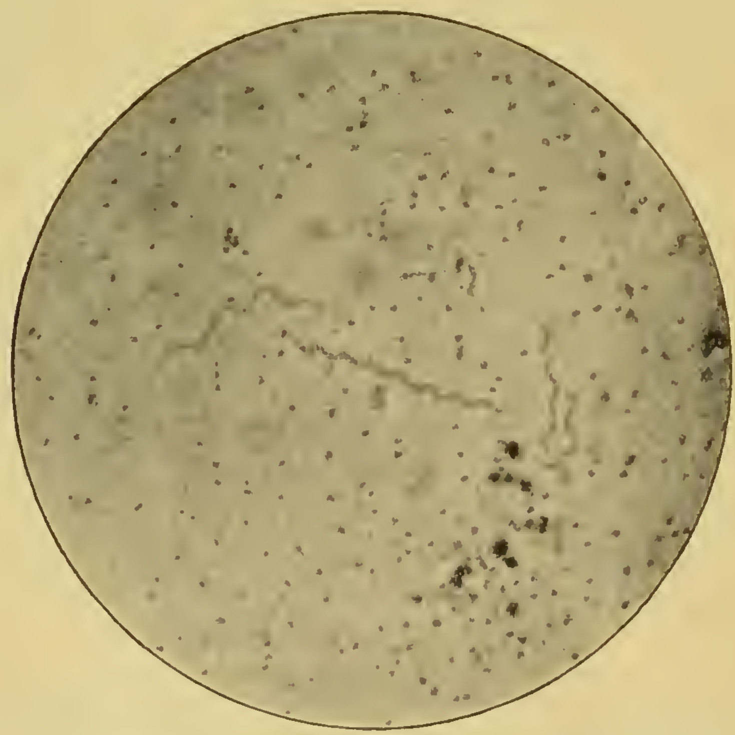

FIG. 2.—Photograph of a film from a syphilitic inguinal gland (Giemsa stain). Three spirochætes are very clearly shown. Mag. ca. 2,800.

(*Cf.* Case 5 of the List.)

(Prepared by Hoffmann. Photogr. by Schaudinn.)

are pointed. On account of the minute size of the object, the investigation of the finer structure, especially of the nucleus, is very difficult and is still incomplete.

The spirochætes bear dilution of the tissue juice with physiological salt solution perfectly well; in one case they still showed movements six hours later. On addition of concentrated glycerine they reacted differently; some became immobile in from five to ten minutes, then remained rigid in the spiral corkscrew-like form for from one to two hours and finally disappeared. Other individuals immediately lost their spiral form and became perfectly straight; the rod then gradually contracted to a short spindle resembling the similar form of the malaria sporozoon; in one case the contraction continued until a short oval form was reached. Such forms were still found after twenty-four hours' treatment with glycerine. It must also be mentioned that such forms, together with the typical spirochæte, were also found in the film preparations.[1]

The cases investigated were the following:—

A.—Cases of Uncomplicated Syphilis.

Case 1.—A. K., woman, age 25, never ill previously. Since January 20, 1905, hard painless nodules on left labium. Eruption and headache dating from about February 22.

Result of Examination on March 3, 1905.—Papulo-squamous syphilide, general indolent swelling of the glands, well marked primary sore of the size of an almond on the left labium majus, and several slightly eroded papules on the surface on the genitals.

This is therefore a case of uncomplicated syphilis of about two and a half months' standing. Before the beginning of mercurial treatment a slightly eroded papule of about the size of a lentil was excised from the right labium on March 3rd, 1905. In the films prepared from the base and interior of the papule, fairly numerous spirochætes of the "pale type" were found (one in every four fields of the microscope).

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[1] Whether we are here dealing with an occasional resting stage of the spirochætes (I know of similar stages in the *S. recurrens* taken from the spleen of patients and from the intestine of the tick) can only be settled by a lengthy comparative study of the developmental history of the different species of spirochætes. My previous investigations upon the spirochætes of birds and of men, which have not yet been published, have, however, shown me that quite similar developmental forms can belong to entirely different developmental stages. One must, therefore, never draw analogies from one form to another.

CASE 2.—M. G., widow, age 58. Previously healthy. Time of infection probably the end of November, 1904. Rash since the beginning of February, 1905.

March 14, 1905.—There is a maculo-papular syphilide, and numerous knotty, raised, and in some cases eroded papules on the genitals. Glands swollen and indolent.

This is thus an uncomplicated case of syphilis, of about three and a half months' duration.

On March 14th, before the commencement of treatment, two papules were excised from the labia. These were scarcely of the size of a lentil, and showed no obvious erosion on the surface. In the films prepared from them a few sparsely scattered spirochætes of the "pale type" were found.

CASE 3.—L., woman, age 25. Previously healthy. Infection probably at the end of November, 1904. In the middle of December painless swelling of the inguinal glands. Early in February, 1905, rash and pains in the neck.

March 15, 1905.—General indolent swelling of the glands, maculo-papular syphilide, papules on the genitals, tonsils and mucous membrane of the mouth.

In this instance also we are dealing with an untreated and uncomplicated case of syphilis of about three and a half months' duration.

A non-eroded knotty papule, of the size of a large lentil, was removed from the labium majus on March 15th for examination. Numerous spirochætes of the "pale type" could be seen in the films prepared from this papule.

CASE 4.—A. K., woman, age 22. Always healthy previously. Probably infected in the middle of November, 1904. In December, hard nodules on the left labium. General symptoms since the middle of January, 1905.

Result of Examination on March 25, 1905.—Universal scleradenitis, maculo-papular rash, specific angina, alopecia, moist papules on the genitals.

This is therefore an uncomplicated case of syphilis, of about four months' duration.

Before commencing the treatment, a somewhat eroded papule of about the size of a lentil was removed for examination on March 21st. An extraordinarily large number of spirochætes of the "pale type" was found in the films (several in each field). Photograph 1 is taken from this case.

Case 5.—W. D., man, age 27. Previously healthy. Infected at the end of July, 1901. Towards the end of August, moist nodules of about the size of a pea were present on the skin of the penis. Shortly after a painless swelling of the inguinal glands developed.

Result of Examination on September 21, 1901.—Typical primary sore on the skin of the penis of about the size of a bean. Lymphatics of the dorsum of the penis indurated. An indolent swelling of the inguinal glands on both sides.

On September 24, at which time the patient had not been treated specifically, the primary sore and an inguinal gland of about the size of a hazel-nut, were extirpated.

This then is a case of recently contracted and uncomplicated syphilis, of about seven to eight weeks' duration, in which no general symptoms had as yet developed.

This case has become of special importance in our investigation. In 1901 Hoffmann had prepared sections of the primary sore and of the gland, and had stained numerous films by very varied methods, but without having succeeded in demonstrating any micro-organisms of any kind. In order to possess material free from all objection, for future examinations he had made a large number of films, and after Schaudinn's first discovery of the spirochæte these, together with the paraffin blocks of the embedded tissues, were handed over for fresh examination. The films had been prepared by smearing small pieces taken from the interior of the gland over cover glasses. In the case of the primary sore the lower half had been divided from the upper ulcerated portion by a clean cut immediately after removal from the body, and pieces from this lower portion were used in making the films. In this way any possible contamination of the preparation with germs from the secreting surface was avoided, even in the case of the primary sore. *In the films, both from the primary sore as also from the gland, numerous spirochætes of the "pale form" were found.* Photograph 2 is prepared from a film made from the inguinal gland in this case.

Case 6.—V. K., man, age 21. Gonorrhœa one year previously, otherwise healthy. Infected on February 25, 1905. In the middle of March two sores developed on the prepuce. Gradual painless swelling of the glands.

Condition on April 3, 1905.—Primary sore of the size of a lentil on the margin of the prepuce, and a second of the size of a pea near the frænum. Non-ulcerating erosion of the glans. Typical indolent swelling of the inguinal glands.

This then is an uncomplicated case of syphilis of five and a half weeks' duration.

On April 3rd both primary sores were excised, and an attempt was made to withdraw some fluid from the enlarged inguinal glands by aspiration with a Pravaz syringe. In films made from the primary sores a few spirochætes of the "pale form" were found. The fluid obtained from the gland contained much blood, and, up to the present, no spirochætes have been found in it.[1]

Case 7.—P. L., man, age 23. Previously quite healthy. Date of infection with syphilis probably October, 1904. First treated (by inunction) at the end of November. Pain in neck since March 20.

Condition on April 4, 1905.—Maculo-papular and annular syphilide. Specific angina, plaques in mouth, moist eroded papule near anus.

In this patient, who had suffered from syphilis for about six months, the secretion from the anal papule when examined in fresh preparations contained a few spirochætes. Stained films made from the papule contained numerous spirochætes of the pale variety.

B.—Cases of Syphilis Complicated by Other Infections.

Case 8.—E. F., man, age 24. Previously healthy. Infected about February 15, 1905. Four to five weeks later a hard swelling appeared at the sulcus. No previous treatment.

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[1] See, however, p. 21 for further investigations on this case.

Condition on April 4, 1905.—A flattened, easily bleeding swelling of the size of a pea with typical indurated base in the sulcus coronarius. Prepuce only withdrawn with difficulty. Balanitis. Moderate swelling of the inguinal glands.

This then is a case of syphilis of about seven weeks' duration complicated by balanitis. Fresh preparations and films were made from the secretion of the primary sore and large numbers of the spirochætes of both types were demonstrated.

Case 9.—M. G., man, age 20. Previously healthy. On March 10, 1905, admitted to the "Charité" with two soft sores and a bubo on the right side. Not possible to determine the date of infection with accuracy. The ulcers healed under iodoform, the bubo was treated by Lang's method. At the base of the chancre, on the inner surface of the prepuce, a typical induration with central erosion gradually developed. The inguinal glands on the left side enlarged, indolent and hard.

We are therefore dealing with a syphilitic sore developing from a soft sore. This was excised on March 28, 1905, and subjected to examination. At this time the soft chancre had completely healed so that the sore now present can be regarded as a purely syphilitic one.

All the films from the excised tissue contained numerous spirochætes of the "pale" variety.

Case 10.—M. K., woman, age 20. Previously healthy. Probably infected in April, 1904. Treatment by inunction from June 20 to July 18, 1904. Again ill from the middle of February, 1905.

Condition on March 3, 1905.—In addition to gonorrhœa there were found, small papillomata and mollusca contagiosa, with numerous raised button-shaped papules on the genitals and adjoining surfaces of the thighs. Syphilitic angina.

This is therefore the first relapse in an attack of syphilis of about one year's duration, which is complicated by a gonorrhœa of long duration, and by gonorrhœal warts and mollusca contagiosa.

Before the commencement of a second course of treatment a papule of about the size of a lentil was excised. The films prepared from the base of the papule contained a large number of different micro-organisms, particularly bacilli, but only a few isolated spirochætes of both types.

CASE 11.—E. W., woman, age 20. Previously healthy. Infection probably in October, 1904. Secreting papules appeared at the end of December. Not treated hitherto.

Condition on March 30, 1905.—Considerable indolent swelling of the glands, specific angina, button-shaped secreting papules on genitals and numerous papillomata.

Here we are dealing with a case of syphilis of about four months' duration which is complicated by gonorrhœal warts. On March 30th films were prepared from the secretion from the papules, and an enlarged hard inguinal gland was extirpated. Numerous spirochætes of the "darkly staining" type and a few of the "pale" variety were found in the films from the papules. The films from the gland only contained a few specimens of the *spirochæta pallida*, none of the easily staining variety (*S. refringens*) were found.

CASE 12.—P. K., woman, age 23. Previously never ill. Infection not determinable exactly. At the end of 1904, headache. Eruption began to appear in January, 1905. Taken into the Charité on February 28, 1905, when the rash, genital papules and inflammation of the glands were seen. No gonorrhœa, no papillomata. After treatment by mercurial inunction all the syphilitic symptoms disappeared.

On April 3, 1905, film preparations of the vaginal secretion were made.

Here we are dealing with the examination of the vaginal secretion of a patient who, at the time they were taken, was free from obvious symptoms. No spirochætes could be found in the films.

C.—NON-SYPHILITIC DISEASES.

CASE 13.—M., woman, age 30. Has never suffered from syphilis, Discharge since December, 1904. Warts on the margins of the vulva since February, 1905.

March 3, 1905.—Urethral gonorrhœa, cervical catarrh, numerous fairly large papillomata. Inguinal glands on both sides of the size of a pea or bean, not especially hard. No signs of specific infection, also nothing exciting suspicion of it.

This then is a case of gonorrhœa with gonorrhœal warts, which may be the cause of the glandular swelling. A few papules were removed for examination on March 3rd. Extremely numerous spirochætes of the "darkly staining" variety were found in the films from the papules.

Lastly, we have made a number of further examinations the results of which we will give quite briefly without entering into a description of the histories. In an uncomplicated case of soft sore no spirochætes were found in the secretion. The vaginal secretion taken from a case of uncomplicated gonorrhœa was examined, but no spirochætes were found. The examinations were also negative in three cases in which films were made from the mucous membranes of the genital organs of syphilitic patients at the time they were discharged from the clinic. Moreover, in the smegma and vaginal secretion of six healthy individuals no spirochætes were found. The control investigations, however, will be extended to further cases, and in a subsequent communication the results of these investigations will be described accurately.

The question now arises whether similar parasites may not have been already described as occurring on the genital organs. At the commencement of our investigations, nothing was known to us as to the occurrence of spirochætes in genital infections. Later Hoffmann found that in the year 1891 Berdal and Bataille[1] had described the constant presence of "spirilla"[2] in cases of balanoposthitis circinata. Csillag[3] had also invariably found these organisms in seven cases of this infection.

According to the investigations of these authors balanoposthitis is contagious and can always be induced by artificial inoculation. It must be especially noted that Berdal and Bataille have only made their inoculations on patients suffering from syphilis, and that the balanitis may be accompanied by lymphangitis and indolent swelling of the glands. The two French authors also state that the "spirilla" are absent, or only present in very small numbers, in other genital diseases. In balanoposthitis circinata, on the other hand, they are always present in very considerable numbers and in sections can be found lying between the epithelial cells. Csillag, who found that the "spirilla" varied between 10 and 20 μ in length and between 0·25 and 0·5 μ in width, and that they stained well with 5 per cent. carbol-fuchsin, showed their presence not only in seven men suffering from balanitis, but also in a few women who had discharges and erosions on the clitoris. He invariably failed to find them in normal smegma.

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[1] Berdal and Bataille. *La balano-posthite érosive circinée. La Médicine moderne*, 1891, p. 340. Abstract in "Ann. de Dermat. et de Syphiligr.," 1891, pp. 981-984.

[2] From the abstracts which alone are at the present time accessible to us, it does not appear certain whether we are here dealing with spirilla or with spirochætæ. According to Csillag's fuller statements the latter is the more probable.

[3] Csillag. "Spirillen bei Balanoposthitis." *Arch. f. Dermatol u. Syphilis*, Bd., 46, p. 150, 1898.

Rona[1] has conducted an exhaustive research upon the occurrence of "spirilla" in genital lesions. In addition to finding them in hospital gangrene and in other necrotic processes, he also found them in the smegma of normal persons, frequently in the secretions of primary sores (in ten out of twenty cases), rarely in the secreting papules of the same disease (two cases out of eleven), almost always in balanitis simplex or circinata, but never in uncomplicated soft sores. He did not make film preparations from syphilitic eruptions. It is noteworthy that he always failed to find "spirilla" in syphilitic lesions of the mucous membrane of the mouth, and that the parasites depicted by him, the systematic classification of which is not determined, appear to be considerably larger than those described by us.

Whether these spirilla have anything to do with the spirochætes described by us in syphilis and gonorrhœal warts, can only be settled by further investigation. At present, we can but state, that **not only on the surface of syphilitic papules and primary lesions, but also deep within the tissues, and within the enlarged inguinal glands**

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[1] S. Rona, "Der gangrenoese, phagedænische, diphtheritische Schanker der Autoren." *Archiv fur Dermatologie und Syphilis*, Bd. 67 (1903), S. 259; and continuation under another title in Bd. 71 (1904), S. 191 and Bd. 74 (1905), Heft 2 and 3. Rona used the terms spirillum and spirochæte interchangeably. He also mentions that Menge-Krönig (1897) had found "spirilla" in normal vaginal secretions.

in cases showing the typical clinical characters of syphilis, characteristic spirochætes are invariably to be found both in fresh preparations and in films stained as described above. Whether it is possible to find definite differences between those parasites found in gonorrhœal warts, and those present in syphilis, can only be determined by further research.

Berlin, *April* 10, 1905.

UPON THE OCCURRENCE OF SPIROCHÆTES IN THE FLUID OBTAINED FROM THE LYMPHATIC GLANDS IN SYPHILIS.

BY

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In a "preliminary note upon the occurrence of spirochætes in syphilitic growths, and in papillomata,"[1] we have communicated briefly the investigations we have been engaged upon during the past few months, which have led to the result that true spirochætes are to be found, not only on the surfaces of syphilitic papules and primary sores, but also in the deeper parts of their tissues, and even in two cases within the specifically affected inguinal glands. These were demonstrated in fresh preparations, and also in stained films using a strong azur-eosin solution. We have expressed ourselves cautiously with respect to the significance of this discovery since we stated that we had also found spirochætes in gonorrhœal warts, and in a case of balanitis. Moreover, Berdal and Bataille,[2] and Csillag, had already shown the presence of "spirilla" in balanoposthitis erosiva circinata, and Rona[3] in different genital affections, even in the smegma of presumably healthy men and women. At that time we already believed that we might conclude that it was possible to distinguish between two kinds of spirochætes, the one coarse and darkly staining, the other much more delicate in structure and extremely difficult to stain. Of these, only the latter could be found in the internal syphilitic growths. In case this suggested difference in variety between the two should finally be substantiated, Schaudinn, who was the first to succeed in discovering them, proposed the name of *S. pallida* for the delicate form, and *S. refringens* for the coarser and more readily stainable variety.

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[1] See preceding paper.

[2] References to the literature are given in the preceding paper.

[3] The "spirilla" of these authors are in all probability spirochætes; Rona employs the terms spirillum and spirochæte interchangeably.

In the meantime we have continued our investigations, and have come more and more to the conviction that we are in reality dealing with two separate forms, and that the delicate variety occurs regularly in syphilitic primary sores, genital papules and indolent enlarged inguinal glands.

In order to obtain material for our work free from accidental admixture with blood, and as far as possible free from all objection, we were not content merely to obtain film preparations from the juices of secreting or intact primary sores or papules, but attempted to get the juice of inguinal glands which showed the typical indolent swelling, because they offered the great advantage for our purpose of being a considerable distance from the primary seat of infection. This was possible in two cases, by extirpation of the inguinal glands, as we have already described. As, however, most patients were not willing to submit themselves to such an operation we attempted to aspirate some of the fluid of the gland by means of a well-fitting syringe of medium size. In this way we obtained on each occasion, without any difficulty, a few drops of juice mixed with a little blood. It contained a few small pieces of adenoid tissue, but could easily be spread in thin layers over cover-glasses.[1]

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[1] The puncture of the glands is effected in the following manner:—The skin in the inguinal region is shaved, disinfected and finally washed with sterile salt solution. A sterilised syringe with an asbestos piston provided with a long needle is then inserted into the gland. The capacity of the syringe is about 5 cc. Care is taken to insert the needle into one of the largest glands. This is then aspirated. If an insufficient

That the fluid obtained by puncture in this way from the inguinal glands must contain the syphilitic virus from the very commencement of the infection, is obvious to everyone who has watched the course of the disease. That, moreover, the virus is present in such a form that it can excite the typical lesions with subsequent constitutional symptoms in non-syphilitic men follows, in our opinion, from the highly important inoculation experiment which was performed by the late Dr. v. Rinecker, and which was published by Bumm.[1] Hence the syphilitic virus must be present in an infective form in the enlarged glands, and therefore probably in the same modification as in the primary sores.

Before we describe the results we have obtained in the eight examinations we have made up to the present, we will give a short description of the form and general appearance of the delicate form of spirochæte. This is the one which stains with difficulty, and has hitherto been regularly found in all the syphilitic inflammatory products at present examined. For this purpose we may refer to the micro-photographs published in our previous paper. Here, too, as on the previous occasion, we will add to the sketch we give of the cases investigated, the result found in the parasitological examination. But as we are now dealing solely with the examination of typical freely movable and enlarged glands, it appears superfluous to separate the uncomplicated cases of syphilis from those complicated with other infections. Every complication is carefully recorded in the protocols.

The *S. pallida*, which hitherto has only been discovered in syphilitic lesions, is an extremely delicate

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supply of juice is obtained, the needle is inserted into the neighbouring glands, so that finally a few drops of whitish-red fluid are obtained. The movement of the gland when the needle is moved proves that the gland is actually punctured. The small operation is practically painless, and no ill results follow. This gland juice appears to be well fitted for inoculation experiments upon monkeys. (Hoffmann.)

[1] Ernst Bumm. Zur Frage der Schanker excision. *Vierteljahrsschrift f. Dermatol. u. Syphilis*, 1882, Bd. 9, p. 285.

object only slightly refractive during life. It is actively motile,[1] and consequently very difficult to see. It has a long, thread-like body which is twisted spirally and pointed at either end. The length varies between 4 and 14 μ, the width is almost unmeasurable, and at most 0·25 μ in the largest individuals. The number of the turns of the spiral varies between six and fourteen. The character of the turns in this variety of spirochæte is distinctive as compared with that of the variety found on the surfaces of the genital organs or within the superficial epithelial layers in many genital lesions. In *S. pallida* they are always numerous, narrow and steep, as in a corkscrew, while in *Spirochæta refringens* they appear flat, wide, and wavelike. Besides the difference in refractive power these spirochætes are characterised, in comparison with all other varieties, by the difficulty experienced in staining them. This is true for all the various stains which have been successfully employed in the case of other species of this general type. Since our previous communication we have again attempted to differentiate this variety from others by staining methods. At present, however, we have only succeeded in staining them sufficiently deeply to enable them to be photographed when using the concentrated Giemsa's solution.[2] (Compare the two photographs given in our first communication). This same staining also serves to bring out the spirochætes in permanent preparations, but it is not easy and requires a well practised eye. In the complete publication of our experiments, which we intend to follow this short communication, we will present a series of microphotographs of those forms of spirochæte which are known up to the present and which we can obtain (*S. obermeieri*, *S. anserina*, *ziemanni*, *buccalis*, *refringens*, the spirochæte of Angina Vincenti, &c.), in order to demonstrate the characteristics of *S. pallida*. The investigations upon the form of the nucleus and upon the resting stage mentioned in our first paper are not completed as yet. In the first place it is essential to establish the existence of the easily recognisable and freely motile form and to determine how frequently this occurs. Further researches must then teach us how long these micro-organisms remain in the motile spirochæte form; whether, as in recurrent fever, regular resting stages alternate with the motile; where and how multiplication occurs, &c. In the first place, it should be determined whether the parasite in the motile stage can be carried over with infective matter into such of the anthropomorphic monkeys as can be infected with syphilis. This experiment, unfortunately, we have not been able to perform yet, as it has not been possible to obtain animals for the experiment.

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[1] The movements seen during life are of the three characteristic forms typical for the species *spirochæta* in contradistinction to *spirillum*, viz.: Rotation about the longitudinal axis, forward and backward gliding movements and flexion of the whole body.

[2] By the kindness of Dr. Giemsa I have received a specimen of his new Eosin-Azur mixture, but have not succeeded at present in staining *S. pallida* more deeply with it. I am much indebted to my assistant, Dr. Gonder, for much assistance in the preparation and examination of the specimens. (Schaudinn.)

We will now give a short description of the cases of syphilis we have examined, to which we will add in each instance the result of the microscopic examination.

Case A (Case 5 in the first communication, see p. 9).—W. D., man, age 27. Previously healthy. Infected at end of July, 1901. At the end of August small moist nodules of about the size of a pea on the skin of the penis. Soon after, painless swelling of the inguinal glands.

Result of Examination on September 21, 1901.—Typical primary sore of about the size of a bean on the skin of the penis; dorsal lymphatics hard; indolent swelling of the inguinal glands on both sides.

On September 24, the primary sore and an inguinal gland of about the size of a hazel nut were extirpated, the patient up to that time not having been treated specifically.

This is a case of recent uncomplicated syphilis of seven to eight weeks' duration, in which the general symptoms have not yet appeared.

This case has become of especial importance for our investigation. Hoffmann had prepared sections of the primary sore and of the gland, as well as numerous film preparations from both sources. He was completely unable to show the presence of any micro-organisms in them, although they were stained in a large number of ways. In order to possess typical material for later investigations, especially for control purposes, he kept the paraffin blocks as well as a number of films, and after the first discovery of the spirochætes by Schaudinn, these were also examined. The films were obtained by drawing small pieces of the gland and of the deeper half of the primary sore, which had been at once separated from the upper ulcerated portion by a clean cut, over the object-glasses. In this way the inclusion of germs from the external surface of the primary sore was completely avoided. In the films from the primary sore, as also in those from the gland, very numerous individuals of the *S. pallida* were found. Other organisms were not to be seen. The spirochætes in both were of uniform and average length, and of the usual number of turns.

Case B (Case 11 in the first communication, see p. 12).—E. W., woman, age 20. Never ill previously. Infection probably occurred in October, 1904. At the end of December moist papules were present. Not yet treated specifically.

Condition on March 30, 1905.—Considerable indolent swelling of the inguinal glands, specific angina, moist, button-shaped papules on the genital surfaces and numerous papillomata.

This is a case of syphilis of about a month's duration, complicated by gonorrhœal warts.

On March 30th, film preparations were made from the secretion of the papules, and an inguinal gland of about the size of a bean was extirpated. Very numerous individuals of the deeply staining form of spirochæte (*S. refringens*), and a few of the non-staining form (*S. pallida*) were found in the films from the papules. The films from the inguinal glands only contained a few isolated individuals of the *S. pallida*. No other organisms were found. The spirochætes in the gland were exceedingly delicate and thin; length on an average only 7 μ, number of turns eight to ten.

Case C (Case 6 in the first communication, see p. 10).—Man, age 21. One year before, gonorrhœa; otherwise always healthy. Infection February 25 1905. In the middle of March two infected spots on the prepuce. Gradual painless swelling of the inguinal glands. Previously untreated.

Condition on April 3, 1905.—Primary sore on the edge of the prepuce of about the size of a lentil. An erosion of about the size of a pea and non-indurated on the penis near the frænum. Typical indolent swelling of the inguinal glands.

This, therefore, is a case of uncomplicated syphilis of five and a half weeks' duration.

On April 3rd the two primary sores were excised, and an attempt was made to obtain some fluid from the inguinal glands by aspiration with a Pravaz syringe. A few individuals of the *S. pallida* were found in the films from the primary sore. The fluid from the gland contained much blood. Careful examination revealed the presence of typical spirochætes in the third film studied. (In the first communication the result of this examination was given as negative).

Case D.—A. S., man, age 21. Gonorrhœa in April, 1904, otherwise previously healthy. At the commencement of April, chancre, which was followed by the development of a painless bubo. Not yet treated.

Condition on April 17, 1905.—Phimosis. Hard swellings on the inner surface of the prepuce can be felt through the skin. No gonococci nor streptococci could be found in the pus discharged from the prepuce. Dorsal lymphatics hard. Inguinal glands, on the left side hard, painless and about the size of a bean; on the right side somewhat smaller, otherwise similar.

This then is a case of syphilis of about four and a half to five weeks' duration, accompanied by phimosis and balanitis.

On April 17th some reddish somewhat milky fluid was obtained by puncture of the left inguinal glands. The admixture of the fluid with blood was fairly considerable. The *S. pallida* in considerable numbers and unaccompanied by other micro-organisms was found in the films (about twenty in each preparation after two hours search).

Case E.—H. H., man, age 28. In childhood tubercular disease of the hip-joint of the right side. Otherwise always healthy. Infection at the commencement of March, 1905. Chancre on the skin of the penis fourteen days later. This never secreted much. No specific treatment up to the present.

Condition on April 17, 1905.—Small, circular, non-ulcerating primary sore of the hardness of cartilage on the dorsal side of the penis three centimetres from its root. The dorsal lymphatics hard and knotty The inguinal glands on both sides, enlarged to the size of a bean or hazel-nut, hard and painless. No rash.

This then is an uncomplicated case of syphilis of about six and a half weeks' duration, with an intact primary sore on the outer surface of the penis.

On April 17th, 1905, the primary sore was excised, and some whitish-red fluid obtained by puncture of the inguinal glands on the left side. Enormous numbers of the *S. pallida*, free from admixture with other organisms, were found in the deeper portions of the primary sore. The individuals were very delicate and thin; on an average 10 μ in length, and with 8 turns. About twenty individuals of the *S. pallida* were found in each film from the gland-juice. These were, on the average, somewhat more active than those from the primary sore, also longer (up to 14 μ), and contained more turns (12 to 14).

Case F.—M. W., woman, age 24. As a child had measles, scarlet fever and whooping-cough, otherwise always healthy. Infection on March 12, 1905. At the end of March had painless swelling of the labia and some discharge.

Condition on April 18, 1905.—Some discharge from the vagina. Gonococci in the discharge from the urethra. Typical induration of the left labium, circular erosion on the inner surface of the left labium (primary sore), characteristic anæmia for the last few days. No eruption.

This then is a case of untreated syphilis, complicated with gonorrhœa, of about five and a half weeks' duration.

On April 18th, 1905, some whitish-red juice was obtained by puncture of the left inguinal glands. Blood preparations were also made. Ten to fifteen individuals of *S. pallida* were found in each film from the gland juice. Length 8 to 10 μ. Number of turns 8 to 12. In the blood taken from the lobe of the ear no spirochætes have at present been found (only two films have been thoroughly examined).

Case G.—Man, age 19. Never ill previously. Infection probably occurred at the commencement of January, 1905. At the beginning of February an ulcer appeared which only secreted slightly, but this yielded to local treatment. Towards the end of February a non-irritant rash appeared. A short time later after renewed coitus several purulent ulcers with increasing phimosis. No specific treatment up to the present time.

Condition on April 19, 1905.—Phimosis, prepuce swollen and hard. some soft sores to be seen. Lymphatic glands on both sides enlarged to about the size of an almond or bean. Annular roseola on the buttocks (old), some psoriasis plantaris, general swelling of the glands.

This is a case of syphilis of about three and a half months' standing, complicated by the presence of soft sores. The glands were completely painless.

On April 19th some whitish-red juice was obtained by puncture of the inguinal glands. The juice was only mixed with a little blood, and up to the present only in two of the films have two very slender and small individuals of the *S. pallida* in its motile stage been found.

Case H.—K. K., man, age 24. Has suffered for the last few months from catarrh of the stomach, is nervous, but otherwise has never been ill. Infection on March 20, 1905. A week later small erosions near the frænum which became hard after fourteen days. Gradual painless swelling of the glands. Not yet treated.

Condition on April 20, 1905.—An infiltration on the right side near the frænum, cartilaginous in hardness, superficially eroded, secreting but little. On the dorsal surface, in the sulcus, a circular erosion with an elastic hard base. Dorsal lymphatics thickened and hard. Inguinal glands indolent and enlarged to the size of a bean on both sides. No general symptoms except anæmia.

This is then an uncomplicated case of syphilis of four and a half weeks' duration, which has not yet been treated.

On April 20th, 1905, some gland juice mixed with blood was obtained by puncture. The *S. pallida* was found in scanty numbers (four to five individuals per cover-glass).[1]

From these protocols it is seen that the *S. pallida* could be demonstrated in the glands of eight undoubted cases of syphilis. In two cases they were found in the extirpated gland, and in six cases in the juice obtained from the glands by puncture. Of these cases, six were from cases of from four and a half to eight weeks' duration, and without secondary symptoms on the skin or mucous surfaces. Two deal with cases examined somewhat later, three and a half to four months. The complications present in four cases, gonorrhœa, gonorrhœal warts, soft sores, balanitis, which have been accurately recorded, appear to us to have no significance for our investigations, since in them also the glands showed the typical painless character of the syphilitic affection, and no organism, with the exception of the spirochætes, could be found.

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[1] It is to be expected that a further careful study of the numerous preparations made will reveal a greater number of spirochætes. The search for these delicate organisms is extremely fatiguing, so that it is not possible to continue it for more than four or five hours a day. (Schaudinn.)

Although we have succeeded in finding in these eight thoroughly examined cases a spirochæte, apparently differing from all previously known varieties, in the fluid of the inguinal glands, yet we are far from expressing a definite opinion upon its possible etiological significance. We content ourselves as in our previous communication with a description of the results hitherto obtained, and must leave for further research the determination of this important question.

UPON THE OCCURRENCE OF SPIROCHÆTA PALLIDA IN SYPHILIS AND THE DIFFERENCES BETWEEN THIS FORM AND OTHERS OF THE SAME FAMILY.

BY

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DEMONSTRATION TO THE BERLIN MEDICAL SOCIETY, MAY 17, 1905.

PART I.[1]

GENTLEMEN, — The investigations upon spirochætes, which, in order to invite criticism, we wish to bring before you in the form of a demonstration of preparations, were commenced by us in February of this year in conjunction with Drs. Neufeld and Gonder, on the suggestion of Dr. Köhler, President of the Imperial Board of Health (*Gesundheitsamt*), and with the co-operation of Professor Lesser.[2] Two smaller communications, the contents of which I may be allowed to state shortly here, have already been published in the *Arbeiten aus dem Kaiserl. Gesundheitsamt*, and in the *Deutsche medicinische Wochenschrift*. On March 3rd of this year I succeeded in demonstrating actively motile spirochætes in the fresh tissue juice of a secondary syphilitic papule removed with all precautions by Dr. Hoffmann. They were very slender and could only be seen under the best objectives. They appeared to differ specifically from the coarser form which not uncommonly occurs on the mucous membranes of the mouth or genital organs. They were therefore termed by me *Spirochæta pallida* to distinguish them from the coarser form, which may for the present be termed *Spirochæta refringens*. The demonstration of the slender, pale form was successfully attained by employing a modification of the well-known Giemsa eosin-azur stain. They were found not only in the deeper layers of primary sores, but also in the specifically infected inguinal glands. Finally, on May 5th, I also found them in the blood drawn by puncture from the spleen of a syphilitic patient the day before the appearance of the rash. The discovery of the organisms in the spleen of a child who had died with congenital syphilis, has also been made by Drs. Buschke and Fischer, and already published by them. My collaborator, Dr. Hoffman, will describe from the clinical standpoint, the methods adopted for obtaining proper material for the investigation, and the results obtained up to the present, as well as the control experiments that have been made. It is my task, as a parasitologist, to depict the morphological features of the *Spirochæta pallida*, and to sketch the points of difference as compared with other previously known varieties of the spirochæte species. In order to render the understanding of these points more easy, I have prepared a number of photographs of different spirochætes in order to demonstrate them to you. A number of original preparations are also put up under microscopes.

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[1] The first part was communicated by Schaudinn, the second by Hoffmann.

[2] The view expressed by several authors that protozoan organisms might play an important part in the etiology of syphilis led to the placing of the search for such organisms in my hands, as Director of the Laboratory of Protozoology of the Board of Health. After communicating with Professor Lesser, he expressed himself as willing to support the work, not only by placing the material of his Clinic at my disposal, but also by asking Dr. Hoffmann, his first assistant, to take part with me

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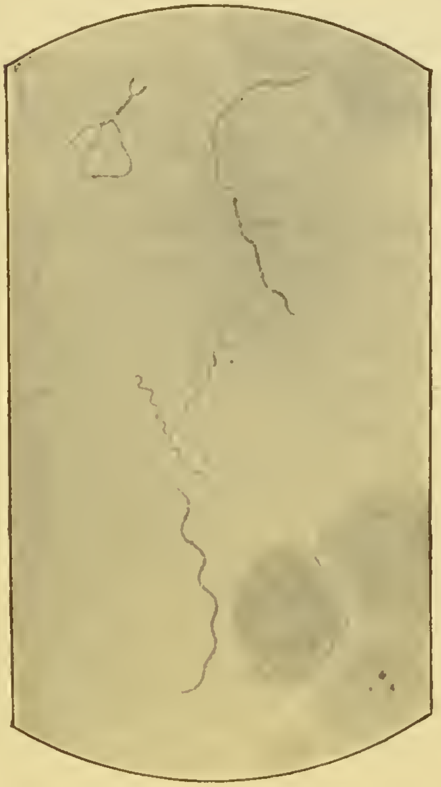
in the investigation. The work of Dr. Hoffmann was to certify to the clinical material, which was the first essential condition for the investigation, and also to examine the various lesions histologically. Dr. Neumann, assistant to the Imperial Board of Health, undertook the bacteriological portion of the work. To him was left the control bacteriological investigations, the culture experiments and other experimental works which have to be conducted later. To me was left the parasitological investigation. My assistant, Dr. Gonder, has been especially valuable to me in this investigation. (Schaudinn.)

I might premise that the typical characters of the spirochætes, the nature of their movement and their structure, place them in a distinct group among micro-organisms, and appear to remove them very widely from the species of *spirillum*. One is therefore incorrect in employing, as has often been the case, the two terms *spirillum* and *spirochæta* for the same object. Whilst I consider, in accordance with the views of bacteriologists, that the *spirilla*, characterised by their stiff and twisted form, belong to the schizomycetes, I have expressed the conjecture, based upon developmental investigations, that the *spirochætæ* being characterised by the possession of an undulating membrane and a flexible body belong to the class of protozoa, a supposition which must be proved for each variety of this species by a study of their developmental history. Up to the present we only know the embryological history of a single spirochæta-like form, the *S. ziemanni*, and must be contented with the conjecture just stated, which perhaps attains a certain probability when we recall the fact that it has not yet proved possible to cultivate the true spirochætes. As with all spirochætes the form of *Spirochæta pallida* is that of a thin, twisted, spiral thread. When living, it moves in a screw-like manner by rotation about its long axis, now in the one direction and then in the other. Also at times one sees undulatory movements, without locomotion, coursing over the whole structure, an expression of the movement of an undulating membrane. Moreover, bending, snake-like or whip-like movements of the whole body occur, so that, unlike the spirillum form, they do not possess stiff, longitudinal axes.

The points of distinction of our form from all other varieties consists in its small size, delicacy, and very slight refrangibility, but above all in the nature of its spiral conformation. One can describe the latter best as corkscrew-like. The turns are numerous, regular, narrow, and deeply inlet. Indeed, this character is quite distinctive, as you may convince yourselves by examining the specimens from very different sources, such as primary sores, papules, glands, spleen, and the hard sores of monkeys. By carefully noting the characteristic form of these spirals, one can, in my opinion, always easily recognise the variety again.



No. 7 of the preparations demonstrated (*cf.* end of this paper). In the middle, two individuals of *S. pallida*, the three others *S. refringens*.

The spirochætes also preserve the shape I have described in stained preparations. Their very slight staining capacity may also be noted as a further distinctive character. By all methods of staining which we have tried the *S. pallida* always appears much paler than any other variety of the species known to me. With most stains it is only very slightly tinged. The best is that mentioned in our first communication, Giemsa's stain. We now employ, for the sake of convenience, the stain prepared commercially by Grübler, and stain with it for one hour only, since Dr.

Giemsa, who has tested the staining capacity of the spirochæte with his mixture, has kindly written to us that the optimum staining is obtained in this time. My collaborators, Drs. Gonder and Hoffmann, have succeeded in making the spirochæte stand out well by prolonged staining with fuchsine and gentian in aniline water, as you will see in a preparation of Dr. Hoffman's.

The essential condition mentioned in our last communication, that in the first place one must determine whether one could carry over the motile stage of the *S. pallida* with the syphilitic virus into such monkeys as are inoculable with syphilis, has, in the mean time, on my suggestion, been fulfilled by Professor Metchnikoff. Through his kindness I am able to demonstrate to you a preparation which Professor Metchnikoff sent to me on May 4th. It is taken from the primary sore of a Macaque monkey on the third day after its appearance, and contains numerous typical individuals of the *S. pallida*. Metchnikoff writes to me that the primary sore is quite dry, is not yet ulcerated, and at present is in its early stage. In a few further cases he has already found our spirochæte, and intends to make a communication upon the subject to the Academy of Medicine in Paris, so that I cannot enter in further detail into the points he has written to me.[1]

PART II.

On account of the very varied forms assumed by the manifestations of syphilis and the extremely changeable and prolonged course which they may exhibit, it is essential, if one wishes to obtain useful results within a reasonable length of time, to choose for investigation only such lesions of the disease as are easily obtainable, and which, as experience has taught, contain the virus in considerable amount. Of these, the most suitable appeared to us to be the primary sores and recent papules on the genital organs, especially if they were intact or only slightly eroded. We have therefore chiefly employed these for our investigations, and up to the present have experimented with seven primary sores, one anal, and eight genital papules. All have been taken before the commencement of treatment with mercury.

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[1] See papers by Metchnikoff and Roux—this volume, pp. 85 *et seq.*

The structures named were carefully excised after thorough disinfection followed by a washing with physiological salt solution. They were then divided by a section at right angles to the surface, and the freshly-cut surface used at once for the preparation of fresh specimens and for stained preparations. These were mounted in the manner described by Schaudinn. By these means we have succeeded in demonstrating the presence of *Spirochæta pallida* in greater or less numbers in all the cases hitherto examined. On the other hand, the coarser form of spirochæte (that named provisionally *S. refringens*) has always been missed in the preparations made from the deeper parts of the syphilitic infiltration. We may especially emphasise the fact that in two non-ulcerating primary sores of the outer skin of the penis very numerous spirochætes of the "pale form" were demonstrated.

In addition to the examination of these, the more usual lesions by means of which the infection is communicated, it appeared to us that the inguinal glands should yield a material especially valuable in our investigation. They become affected early in the course of the disease in a very characteristic form as indolent buboes, and since they are placed a considerable distance from the original seat of infection, accidental contamination would only reach them with difficulty. That they do contain the virus is quite clear to everyone conversant with syphilitic processes. That, moreover, they contain it in the same form as does the primary sore and moist papules, follows from a very important experiment conducted by the late Dr. v. Rhinecker, and published by Bumm, the result having also been confirmed by Neisser on monkeys: About eighteen days after the inoculation of the juice expressed from a freshly excised syphilitic inguinal gland, three typical primary sores appeared. After a further period of thirty-four days, *i.e.*, fifty-two days after inoculation, a typical syphilitic eruption appeared, accompanied by a characteristic rise of temperature.

In two instances we succeeded in getting fresh inguinal glands from recently infected patients, and were able to demonstrate the spirochæte in the films made from them. One of these two cases—the first in which the demonstration of the spirochæte in the inguinal glands succeeded—had been operated upon by me in the year 1901, and the material then obtained treated by our present methods.

In order to obtain the gland juice in other cases I have punctured enlarged inguinal glands, using the method recently described by me, and have thus obtained in every case a few drops of gland juice. This fluid contains small pieces of adenoid tissue more or less mixed with blood. It spreads easily over a cover glass, forming a thin film, and stains well. How rich this fluid is in lymphocytes, characteristic cell fragments and blood platelets, one of the preparations demonstrated will prove to you.

By means of this method we have been able, without fail, to find the spirochæte in the affected inguinal glands in all of the ten cases, though on several occasions only after a prolonged search. Other micro-organisms have never, up to the present, been found accompanying them. On the whole, therefore, we have proved so far the presence of the spirochæte in twelve typically infected syphilitic inguinal glands.

Latterly we have also attempted to extend the observation by proving the presence of the spirochæte in the blood and in the blood-stained fluid obtained by puncture of the spleen in patients at the time of the eruption. As you have already heard, Schaudinn has succeeded in finding and staining quite typical specimens of the spirochæte in the splenic blood. Reckzeh, whom we asked to undertake the extremely difficult and tedious task of examining the blood, has succeeded in demonstrating spirochætes in blood obtained from the finger, but as they were somewhat deformed it was not possible to identify them with certainty.

Buschke and Fisher, to whom I had demonstrated my preparations, have obtained exceedingly numerous spirochætes of the pale form in specimens prepared by our method from the liver and spleen of a child who had died from congenital syphilis. They have kindly lent us one of their preparations that we may photograph and demonstrate it.

Finally, it appears to us to be of special significance that Metchnikoff has been able to prove the presence of the same spirochæte in the lesions produced by the inoculation of monkeys with syphilis, as you will be able to see in one of the specimens exhibited to-day.

Such are the positive results hitherto obtained, and it only remains to add a very brief account of a few control investigations. In doing this it will not be necessary to repeat the data given in our first communication.

We have been unable to demonstrate spirochætes in film preparations obtained from the bubo due to a soft chancre. The same is true for films made from cancerous, sarcomatous or lupoid tissues. We found the coarse spirochæte already described by Berdal and Bataille in the smegma of a patient suffering from balanitis erosiva circinata, whilst the juice obtained from the swollen inguinal glands of the same patient were quite free from them. In a second patient who had contracted syphilis ten years previously, but for the last nine years had been completely free from all symptoms, and showed not the least sign at the present time, but was suffering from balanitis erosiva, we also found the inguinal glands quite free, although numerous spirochætes of the coarse variety were present on the penis.

It might appear advantageous to enter into a discussion of the close analogies which exist between syphilis and relapsing fever, and to trace out the conjecture whether the delicacy of the spirochæte might not be regarded as a special adaptation to the restricted spaces of the lymphatic system, which are so characteristically the seat of syphilitic processes.

We think it best, however, not to enter further into such a consideration, although it might have a certain value as tending to incite further extensions of our researches, and we may therefore be content to reaffirm the conclusion at which we have arrived in our last communication :—

In addition to the most important and invariable discovery of the micro-organism in seven primary sores, nine secondary papules, and twelve typically infected inguinal glands, it has also proved possible to demonstrate their presence in the splenic blood, once in the liver and spleen of a child that had died of congenital syphilis (Buschke and Fischer), and lastly, Metchnikoff has given the important proof that they occur in the primary lesions following the inoculation of syphilis in monkeys. Still, for the present, we are unable to make a definite statement as to the etiological significance of this hitherto unknown minute organism. We simply content ourselves with a statement of the results we have obtained.

At the conclusion of the discussion the following preparations were demonstrated :—

(1) *S. pallida* from several primary sores.
(2) The same from secondary papules.
(3) The same from syphilitic inguinal glands.
(4) The same from the fluid obtained by puncturing the spleen of a man recently infected with syphilis.
(5) The same from a film taken from the spleen of a 10-weeks' old child who had died from congenital syphilis (Buschke and Fischer).
(6) The same from a primary sore of a Macaque inoculated with syphilis (Metchnikoff).
(7) *S. pallida* and *S. refringens* in a preparation from a genital papule.
(8) *S. refringens* from a papilloma.
(9) Spirochætes from a case of balanitis erosiva circinata.
(10) *S. buccalis.*
(11) Spirochæte from Vincent's Angina (lent by Dr. Börger).
(12) *S. anserina* (goose septicæmia).
(13) Spirochæte of the so-called fowl spirillum fever.
(14) *S. obermeieri* (Recurrens).

ADDENDUM BY DR. ERICH HOFFMANN.

Since the printing of the above communication Levaditi (*Semaine medicale*, May 24, 1905, pp. 247-248) has announced that he has found numerous specimens of the spirochæte in the fluid and scrapings of a bulla of an eight-days' old child suffering from syphilitic pemphigus. Further, that in a second case, that of a child of two months who had died of congenital syphilis, he had found the spirochæte in the spleen, liver and lung. He also mentions the fact that Salmon has found the spirochæte, practically as a pure culture, in the fluid taken from a pemphigus bulla in a child suffering from congenital syphilis.

I can confirm and extend these results from the examination of a case of severe congenital syphilis kindly sent to me by Dr. Bumm.

The case was one of a child who had died ten hours after birth, and in whom, in addition to pemphigus, a very marked swelling of the liver and spleen was found. Seven to eight hours after the death of the child I could demonstrate the presence of the spirochætes in special abundance in the pemphigus fluid and in the liver, which was also histologically altered in a typical manner. They were also found in smaller number in the spleen and enlarged inguinal glands.

Lastly, Schaudinn and I have been able to confirm the statements of Metchnikoff and of Wechselmann that the spirochæte may be present in its typical form in secondary papules in which the surface is quite intact, and where, too, the papules are far removed from the genital organs —in our case on the breast and back.

FURTHER REMARKS UPON THE OCCURRENCE OF SPIROCHÆTA PALLIDA IN SYPHILIS.

BY

ERICH HOFFMANN.

READ BEFORE THE GESELLSCHAFT DER CHARITÉ-AERZTE ON JUNE 8TH, 1905.
(From the *Berliner Klinische Wochenschrift*, No. 32, 1905.)

Gentlemen,—In three papers Schaudinn and I have reported upon the occurrence of a hitherto unknown variety of spirochæte, characterised by a slender, slightly refractive body with corkscrew-like spirals, and possessing but a slight power of taking up stains. These may be found not only on the surface of syphilitic lesions, but also in the deeper parts of their structures. By the delicacy of their structure, the form of their spirals, and the difficulty of staining them, this variety of spirochæte, termed by Schaudinn *Spirochæta pallida*, differs from the larger form which has long been known though little studied, found in Vincent's angina, balanitis, papillomata, gangrenous ulcers, and on the mucous membranes, and occasionally in the secretions of the genital organs of healthy persons. In our last communication we stated that we had succeeded in demonstrating the *S. pallida* in seven primary sores, nine secondary genital and perigenital papules, twelve inguinal glands and once in the splenic blood obtained by puncture. At that time, too, we were able to state that Metchnikoff had found the *S. pallida* in the four primary sores and in four intact secondary skin papules situated far from the genital organs in a monkey that had been inoculated with the virus, and that Buschke and Fischer had found numerous spirochætes of this type in the liver and spleen of a child who had died from congenital syphilis, the *post mortem* having been made thirty-six hours after death.

In the discussion which followed our communication, a number of confirmatory observations were given. Buschke stated that he had also found spirochætes in the blood taken during life from the case above mentioned, but added that it might well be but an interesting observation, having nothing to do with syphilis and simply due to the occurrence of sepsis. Pielicke also stated that in conjunction with Frosch he had succeeded in finding the spirochæte in film preparations from primary sores, from a papule and in the inguinal glands. Further, Wechselmann and Lowenthal communicated a number of positive results from the examination of intact secondary papules. In one case they found nine spirochætes within the protoplasm of a large cell. Finally, Reckzeh confirmed the occurrence of *S. pallida* in syphilitic buboes. When examining numerous preparations of blood taken from patients who had recently contracted syphilis, he had on one occasion only been able to find structures which might possibly represent degenerative forms of the spirochæte. I need not discuss the quite untenable suggestion, which was refuted by us and others, that the spirochæte might have been added with the staining reagent (Giemsa's stain).

In the mean time, a further series of confirmatory observations have appeared, which I may discuss briefly before I turn to my own recent results. In the first place, Bordet has stated that, in conjunction with Gengou, in the year 1903, he had once stained a slender form of spirochæte in the deeper parts of a chancre and in a papule from the mouth. He could not find it again on further investigation, and therefore laid no special significance upon it. Soon after, Paschen stated that he had succeeded in demonstrating the spirochæte in primary sores, and subsequently Jacquet stated that he had found them in some cases of secondary syphilis. Of especial importance are the results of two other French observers, Levaditi and Salmon, who were able to find the spirochæte in cases of congenital syphilis. Levaditi has investigated three cases. In the first he found the spirochætes in the fluid taken from the pemphigus bullæ of a child born with congenital syphilis, as well as in the non-ulcerated papules. The second case was that of a child, who died fifty-three days after birth, where numerous spirochætes were found not only in the skin papules, but also in the liver, spleen and lungs. In the third case, that of a child born with syphilitic pemphigus and dying a few hours later, the spirochætes were found in the fluid from the bullæ, in the liver, and in the spleen. As the result of his investigations Levaditi, following Metchnikoff, comes to the conclusion that in all probability syphilis is a chronic " spirillosis " caused by the *S. pallida*. Salmon describes the finding of numerous specimens of the spirochæte in the fluid from pemphigus bullæ, but failed to find them in the mucus from the nose or in the blood.

Kraus, Volk, Ehrmann and Oppenheim,[1] publish most valuable confirmation. Kraus stated that almost without exception, he had been able to find spirochætes in syphilitic primary sores, papules of the skin, and lymphatic glands, but that he had always failed to find them in control preparations. In smegma, in balanitis and in papillomata, he had often found many examples of a delicate form of spirochæte which was very similar morphologically to the *S. pallida*, but could always be distinguished from it since it stained more easily. Paltauf pointed out the great significance of this discovery and expressed the view that the spirochætes were protozoa. Volk described the results obtained in investigations in Lang's Clinic, in which he had succeeded as a rule in finding the spirochæte in primary syphilitic lesions and in papules. In syphilitic inguinal glands on the other hand he had failed. In a very extensive series of control examinations, particularly upon non-syphilitic lymphatic glands, he had never been able to find the spirochæte. Ehrmann and Lipschutz have also found the spirochæte in papules from the genital organs, skin and tonsils, but never in control preparations. Finally, Oppenheim also described confirmatory results from Finger's Clinic.

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[1] May 26, 1905, in the *k. k. Gesellschaft der Aertzte* in Vienna.

In conclusion it may be mentioned, that recently Döhle has described the presence of spirillum-like structures in the secretion from chancres, and especially in the blood of syphilitic patients. According to the figures he gives, these bodies, which are found in fresh specimens and only in very small numbers in the blood, are certainly not identical with the *S. pallida.*

Now that I have referred briefly to all the publications which have appeared upon the subject, I will turn to a short account of my own investigations which have been continued in the meanwhile. The method employed has been the same, though recently I have almost exclusively used the stain prepared by Grübler or Leitz according to Giemsa's formula. Ten to fifteen drops of this mixed with 10 cc. of distilled water are poured over the cover glasses, which are placed face downwards in a porcelain basin. After about an hour the staining is quite sufficient.

I have frequently obtained specimens containing the pale spirochætes in very considerable numbers from a number of primary syphilitic sores and genital papules, both in the secretion as well as in the scrapings from the tissue. The spirochætes appear to be least numerous in teased preparations, where accompanying them one may find bacteria and not uncommonly spirochætes of larger varieties in considerable numbers. The spirochætes are found more abundantly if the eroded surfaces are first thoroughly cleaned and then well rubbed with a stiff platinum loop until an abundant supply of serum mixed with blood appears. They are obtained in greatest number, as has already been said, by scraping the tissue with a sharp spoon, care being taken not to include too much blood in the film. I have now found the *S. pallida* in extra-genital primary sores (lips and chin) on several occasions, usually in large numbers. I have also succeeded in finding them in the scrapings from some papules on the dorsum of the tongue, and my colleague, Dr. Roscher, has obtained them from papules between the toes.

I have also been able to prepare specimens containing spirochætes in greater or less number from the fluid aspirated from lymphatic glands, more particularly when I have massaged the punctured gland and moved the point of the needle within the substance of the gland during the aspiration. I have usually ejected the aspirated juice into a small capsule, and have then selected those parts most free from blood for the films. Often the final drops expressed from the syringe by forcible emptying have proved to be the richest in spirochætes. In this way I have been able to demonstrate them not only in the juice obtained in a great number of cases from the inguinal glands but also in one case (that of a primary sore on the chin), from the submental glands. In addition to the spirochætes I have always found accompanying them a number of characteristic small spherical bodies which stain blue with the Giemsa stain. They are most abundant in the juice from lymphatic glands, rarer in sclerosed parts or papule secretions, and appear to have arisen by budding from endothelial cells or perhaps from leucocytes. In fresh preparations they often show active amœboid movement. I am not able to say anything further upon their significance. It is quite easy to distinguish them from blood platelets which are present in large numbers but which stain red with Giemsa's reagent.

I have also succeeded, as has my colleague Dr. Roscher, in finding the spirochætes in many instances in secondary papules upon the skin of the breast and back. Numerous spirochætes were found in a freshly developed spot on the back in a case with marked papular syphilide three months after infection. In a case showing a varicelliform syphilide three and a half months after infection, specimens containing large numbers of the spirochæte were prepared from an intact pustule. Neither of these cases had been treated with mercury. In one patient who had become infected eight months previously, and had already been through two courses of treatment with mercury, a few extraordinarily short individuals were found in a papule on the skin of the back.

The results found in a case of severe congenital syphilis, which Dr. Bumm had most kindly sent to me for examination, appear to me to be of special importance. The child was born on May 24th, 1905, the mother, a waitress, showing most typical secondary symptoms. It died, without having taken food, ten hours after birth. During life it had shown obvious signs of syphilis, such as pemphigus bullæ on the palms of the hands and soles of the feet, as also a marked enlargement of the liver and spleen. The examination was commenced seven to eight hours after death, but it was not possible to examine all the organs at once. The liver and spleen were enormously enlarged and tough. The inguinal glands, as also those in other positions, were slightly enlarged. Histological examination of the liver revealed a very marked interstitial cellular hepatitis. Also, in the spleen and the inguinal glands frequent accumulations of round cells around the blood-vessels were to be found. Spirochætes were found in large numbers in the film preparations made from the liver; in those from the spleen in fair numbers; and in those from the inguinal glands in scanty numbers. They were also very abundant in the films made from the bases of the pemphigus bullæ. I could not find any in the bone marrow (only one film was made from this material).

The spirochætes found in the skin bullæ and in the different organs from this case of severe congenital syphilis which was examined soon after death, correspond exactly in form and staining qualities with those found in so many cases by Schaudinn and myself in the acquired syphilis of adults.

In the last place I might emphasise once more the fact that though by ourselves and others a fairly considerable amount of control material has been examined, no one has been able to find the *S. pallida* anywhere except in syphilitic lesions. It is true that in balanitis erosiva in addition to the great number of the large spirochætes, a few more slender individuals occur, but they are easily to be distinguished from the *S. pallida* by the nature of their spirals and the ready manner in which they take up the stain.

If I now sum up the results obtained by ourselves and others, we find that the *S. pallida* has been found :—

(1) In the syphilis of adults: (*a*) in genital and extra-

genital primary sores; (*b*) in genital and perigenital papules, both secreting and intact; (*c*) in moist papules between the toes; (*d*) in papules and pustules on the skin far removed from the genital organs; (*e*) in the papules on mucous membranes (tongue, tonsil); (*f*) in lymphatic glands (inguinal and submental); (*g*) in splenic blood obtained by aspiration; (*h*) in blood taken from the ball of the finger. (?)

(2) In congenital syphilis: (*a*) in the pemphigus bullæ and skin papules; (*b*) in the liver; (*c*) in the spleen; (*d*) in the lymphatic glands; (*e*) in the lungs; (*f*) in the blood.

(3) In the primary lesions of monkeys that have been experimentally inoculated.

According to the careful investigations of Reckzeh, which have been conducted upon the patients of our clinic, shortly before the appearance of the eruption, during the eruption, and later, the *S. pallida* only appears in extremely small numbers in the blood. Also in the splenic blood obtained by puncture, Schaudinn could only find a few isolated individuals after a prolonged search. It is to be expected from the experiments which have been made upon man in which blood from syphilitic patients has been injected, that the syphilitic virus can only be present in small amounts, since a positive result only appears to have been obtained by the employment of large quantities of blood. Should it subsequently prove that the *S. pallida* is the exciting cause of syphilis, it would then follow that they do not find favourable conditions for their development in the blood stream and therefore soon after gaining it leave the vessels and pass into the perivascular lymph spaces, where, as is well known, the earliest histological changes are to be found. Thus their delicate structure and minute size would receive a complete explanation as an adaptation to the narrow spaces of the lymph channels which are more especially the seat of syphilitic lesions, whilst the coarser and larger size of other varieties of spirochæte would appear adapted to the wider spaces of the blood capillaries.

Added during Correction (August 30, 1905).

Since the above was written I have succeeded in finding the spirochæte on many occasions in secondary syphilis (breast, back and arms). I have again found them in the pemphigus fluid taken from a child suffering from congenital syphilis, and in another case of a child dying soon after birth from congenital syphilis in the pemphigus fluid, spleen, lung, suprarenal, and in a gummatous growth in the liver. In two other cases of congenital syphilis examined at an earlier date the examination proved negative.

Finally, I might mention that Dr. Mulzer has found spirochætes collected into thread-like masses (skeins of twenty to forty individuals) in a recent primary sore. Dr. A. Alexander had previously demonstrated similar skeins to me in the scrapings from syphilitic papules on the throat. I have never been able to find spirochætes which were not readily distinguishable from the *S. pallida* in any other diseases or in healthy persons, although a large number of control examinations have now been made. I have never found spirochætes in the inguinal glands except in syphilis, even in cases in which numerous spirochætes were present on the ulcerated surface of the penis, and although the balanitis circinata produced by their presence had led to distinct enlargement of the glands.

THE SPIROCHÆTA PALLIDA.

BY

FRITZ SCHAUDINN.

(*From the Deutsche Medizinische Wochenschrift*, 1905, *No.* 42,
19 October, 1905.)

THE literature upon the *Spirochæta pallida* has increased to an unusual extent in the few months that have elapsed since the appearance of the first communication dealing with the occurrence of this organism in syphilitic lesions. In spite of its small size and the difficulty experienced in demonstrating it, this spirochæte, which, however, is well characterised by its general appearance, has already been found in the most varied syphilitic lesions by more than a hundred observers. The finding of these organisms will be extended yet further as investigators become more practised in distinguishing and staining them. Most observers state, that at the beginning of their work they only succeeded in finding a few isolated individuals, or obtained negative results, but on further practice always obtained more numerous positive results. This has also been the case in my own instance. Since I have been engaged in the study of this organism the number of positive results has gradually risen, until at last I have been able to demonstrate its presence in all the cases (over seventy) of primary and secondary syphilis which have been at my disposal. I have now become convinced that the organism occurs regularly in all forms of syphilis up to the tertiary stage, but in this I have uniformly failed to find it up to the present. I expect, however, that in this late form of syphilis the spirochæte will be found in the less characteristic granule-like resting stage, which I have described briefly in my first publication with Hoffmann. Later, when I have examined the tertiary products more extensively, I shall be able to return to this question. I do not intend for the present to discuss the finer structure, the nuclear arrangements and the developmental stages of the spirochæte, because the investigations upon these points are very difficult and still far from completion, on account of the small size of the organism. To communicate isolated observations, conjectures, or ideas at this time seems to me to have but little value. In my opinion we shall only be able to make further progress after the completion of comparative and preliminary experiments upon larger varieties of spirochætes. We do not know the development of a single true form of spirochæte. The form termed by me *S. ziemanni*, which, at the time, I thought comparable with other spirochætes, only possesses a spirochæte form for a short stage in its developmental history. My later comparative researches convince me more and more surely that this organism is, in reality, far removed from the typical spirochætes (*S. plicatilis*, *obermeieri*), and probably only possessed phylogenetic relationships to them at some very early period in the history of its evolution. Just as the general plan of structure of a trypanosome (nuclear and motor apparatus) actually occurs in different groups of protozoa as a transitory stage in their development (somewhat comparable with the gastrula stage in the metazoa), so also might the spirochæte form occur occasionally as a morphological type in the development of the protozoa and indicate phylogenetic relationships, whose importance we can scarcely estimate correctly in the present state of our knowledge. Such problems as these will, however, stimulate us to further researches; they are, moreover, worthy of discussion and deserve to be thoroughly studied by those protozoologists who are working in the domain of experimental medicine, so that they may not lead to false ideas upon the value of theoretical speculation in the study of the protozoa.

The very active investigations upon the *S. pallida* have brought out a few other difficulties which are of a more practical nature and which I might now briefly dwell upon. It appears that spirochætes are much more widely distributed in many pathological lesions, especially in disintegrating human tissues, than has been usually supposed. Just as with the trypanosomes, it is not easy to find points of distinction between these organisms, since they possess but few anatomical characteristics. Hence some authors have found difficulty in distinguishing the variety described as *S. pallida* from other forms which are found in different infections.

By comparative investigations one soon finds that *S. pallida*, which occurs in pure syphilitic products while all others are absent, only possesses very slight degrees of variation, and in contradistinction to all other forms of spirochæte is very readily distinguished.

The discrimination from other forms is, with a little practice, effected most easily with the living organism. The delicate body and the slight refractivity of the *S. pallida*, combined with the characteristic form of the spiral with its narrow, deep, uniform, and generally numerous turns (ten to twenty-six) can scarcely be confused with any other object. The chief point, however, is that with the living object one can see that the organism shows this typical spiral form not only while in movement but also while at rest. All other analogous organisms, on the other hand, only show the typical spiral form when in the most active movement, and when at rest return to a slightly spiral form approximating to a straight line. The typical stiff, one might say lathe-turned, appearance of the *S. pallida* depends upon the fact that the spiral is in its case preformed, and only occasionally lost through injury, whilst the other types only form narrow spirals by active rotation, and straighten out again on coming to rest.

In permanent preparations the difficulty arises that on the drying of the films some of these spirochætes may be caught at the moment of most active movement and may therefore appear much twisted. For those who have little experience with them, it may then be difficult to distinguish them from the *S. pallida*. In order to come to a conclusion one must then make use of the other less characteristic differences. In the first place we may compare them with typical pale forms taken from undoubtedly pure syphilitic cases, examining the thickness (best microphotographically), the number of turns (other spirochætes never reach the number of ten to twenty-six), the depth of colour on staining and the tint (*S. pallida* is coloured pale red with Giemsa's stain, other forms bluish), and lastly, the shape of the ends (spirochæta has sharp pointed ends, the pseudopallidæ from ulcerated carcinoma for instance, blunt rounded ends). In this way I have always been able, even in stained preparations when only a few individuals were present, to determine definitely whether *S. pallida* or some other form was the one present. As is to be expected, all kinds of diverse appearances may occur, due to the manipulation necessary during the preparation of the film; for instance, one may find in specimens of *S. pallida* individuals with flattened turns, or straightened out, or with blunt ends. In all such cases general appreciation of what is typical is of course necessary, and one must not expect to find in the one individual all the typical characters of the species. In spite of all, I do not doubt that there may occur cases in which one cannot arrive at a sure conclusion; and indeed, such difficulties not uncommonly occur with much larger objects.

The difficulties in distinguishing the *S. pallida* from other varieties increase when the films are insufficiently stained, because then the commoner spirochætes appear pale and much more delicate than in correctly stained preparations. I have now tested the other stains which have been recommended for *S. pallida*, but can still only recommend the new Giemsa's stain as the best. One must proceed exactly according to the directions given by Giemsa. If they have been properly stained for an hour, alkali having been added, the spirochætes will stand out distinctly red, and the nuclei of the leucocytes will appear a dark blackish-red. If the latter are blue the staining has not been successful. In order to avoid artefacts as far as possible, I always use osmic acid vapour for the fixing of the film preparations. The turns of the spiral and the pointed ends are better preserved by this method than when they are fixed by the ordinary method of drying. The staining does not appear to suffer at all if they are only exposed for an instant to the osmic vapour.

For bringing out the locomotor organs of the different spirochætes it is best to employ the old Löffler's dye for the flagella of bacteria. For this purpose one must lay special stress on the importance of thin films, otherwise the dark colouring of the surrounding parts will conceal the structure of the spirochæte. By using this method I have been able to bring out the undulating membrane of the spirochæte so clearly that I could photograph it, and gradually have so practised the eye to the recognition of this structure that I can now differentiate it without any difficuly both in preparations stained with Giemsa's stain or in fresh specimens.

At the time the first communication was published I had not been able, in spite of many attempts, to see the locomotor organs at all clearly. I found no flagella at the ends, but believed that I could perceive signs indicating the presence of an undulating membrane. I saw, for instance, in organisms at rest, wave-like movements coursing along the spiral.

With Löffler's stain I studied other forms of spirochætes, and in all (*S. dentium*, *refringens* from gonorrhœal warts, spirochætes from ulcerated carcinoma and other ulcers of the skin) found the undulating membrane so clearly differentiated that I could easily photograph it. In my complete paper I shall show these different points in a series of plates; it is not possible to reproduce the negatives as text figures at all successfully, so for the present, I can only refer to the drawings given in figs. 3-5. The periplast, in the form of a spiral hyaline layer, surrounds the axis of the organism which stains a deep black-red colour with Löffler's stain and contains the nuclear apparatus within the entoplasm. It was interesting to compare with this type of spirochæte, the free-living form, *S. plicatilis*. I found, as

I will show later, that this large form, just as the previously mentioned variety, possesses no flagella. The ends are bluntly rounded off, the periplast forming, however, a beautiful undulating membrane (fig. 7). The nucleus appears as a long thread-shaped structure running along the axis of the organism, and might represent the locomotor nuclear apparatus of the trypanosomes, whilst the vegetative nuclear mass surrounds this thread in the form of granular chromidia. Perrin has figured a very similar arrangement for the nuclear apparatus of the *Trypanosoma balbiannii* of the oyster, which in consequence one might better place in the family of the spirochætes. Also in *S. refringens*, *dentium*, the spirochæte of Vincent's angina, of ulcerated carcinoma and of similar degenerative processes, the locomotor organs in the nuclear apparatus appear to me to correspond to that seen in *S. plicatilis*. None of these forms possess flagella. In contradistinction to them the examination of *S. pallida* by Löffler's method showed no trace of an undulating membrane, though at each end a long delicate flagellum could be clearly seen (fig. 1). Whether an undulating membrane is also really present, but very difficult to demonstrate on account of the minute size of the organism, as my observations upon the living organisms have always suggested, can only be determined by further work, and after the eye has become more acute in detecting it. At the present time, it appears to me that the *S. pallida* is circular in transverse section and does not possess the band-shaped form seen in all other varieties of spirochæte studied. The periplast appears to me to be developed uniformly all round the organism, thinning out at the two ends to project as the two flagella, the length of each being about that of four to six turns of the individual spirochæte. After I had first clearly seen these flagella in successful Löffler preparations, and had accustomed my eye to their recognition, I also found them in my Giemsa specimens if they were sufficiently thin, and can now recognise them in the living state, although at first I had sought for them there without success. I will illustrate the arrangement of the flagella by means of microphotographs in my later paper.

I have repeatedly observed individuals of the *S. pallida* possessing two flagella at one end, and these were for the most part short and thick individuals (fig. 2). It is not improbable that such stages appear before longitudinal subdivision, and that just as with the trypanosomes, this doubling of the flagella precedes this process. I will go into this question more thoroughly at a later time. This short communication only aims at pointing out to those working upon these organisms the new and important sign

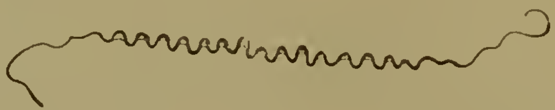

FIG. 1.—*Spirochæta pallida* with flagella at each pole.

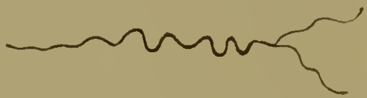

FIG. 2.—*Spirochæta pallida*; smaller, thicker example, showing two flagella at one pole (longitudinal division ?).

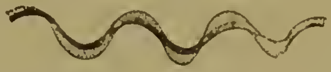

FIG. 3.—*Spirochæta refringens* with distinct undulating membrane from a condyloma.

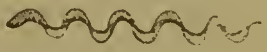

FIG. 4.—A smaller, closer-rolled example of the same.

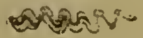

FIG. 5.—*Spirochæta* from an ulcerated carcinoma; note the blunt rounded end and the undulating membrane.

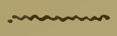

FIG. 6.—*Spirochæta dentium*; the smallest individual of this species which I have observed; many spirals.

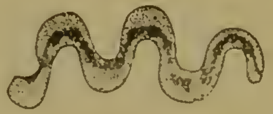

FIG. 7.—*Spirochæta plicatilis*. End of a long individual.

by which we can differentiate the *S. pallida* from all other forms of spirochæte hitherto described, viz., the presence of flagella. I must emphasise the fact that I have only found flagellated spirochætes of the close spiral variety in syphilitic lesions, and I consider this very characteristic form as specific.

As, according to this discovery, the *S. pallida* differs, not only from the general types of spirochætæ and spirilla, but also from all other known members of this family, I agree with the suggestion brought forward by Vuillemin[1] that this form should be placed in a separate category, for which he has suggested the generic name "*spironema.*"

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[1] Vuillemin, "Sur la dénomination de l'agent présumé de la syphilis," *C. R. Aca. Sci.*, *Paris*, vol. cxl., June 5, 1905.

SPIROCHÆTA PALLIDA IN A MACAQUE INOCULATED WITH BLOOD FROM A CASE OF SYPHILIS.

BY

ERICH HOFFMANN.

(From the *Berliner klinische Wochenschrift*, November 16, 1905, No. 46.)

While more than a hundred communications describing the occurrence of *Spirochæta pallida* in the most diverse lesions of recent syphilis in man have already appeared, the number of researches which refer to the discovery of the organism in experimentally induced syphilis of monkeys is still quite small. So far as I have been able to examine the literature, only Metchnikoff, R. Kraus, Zabolotny and Schaudinn have described such results, and apparently they have only employed syphilitic papules or primary sores from man or from inoculated monkeys for purposes of inoculation. I have now succeeded in proving the presence of *S. pallida* in the primary lesions of a macaque monkey which had been inoculated with the blood of an untreated man who had contracted syphilis at least six months previously. The history of the patient from whom the blood was taken is briefly as follows :—

A man, aged 25, who had always been healthy previously. A soft sore formed on the prepuce in June, 1904. This healed without further result within three weeks. At the commencement of July the patient noticed several circular inflamed spots which secreted pretty freely. Soon after similar secreting spots developed on the scrotum, anus and the skin of the abdomen. Headaches occurred at night-time. In spite of this the patient remained untreated up to his admission into the clinic on October 11th, 1905. At this time a number of closely set papules were present on the penis, scrotum, anus, abdominal wall in the neighbourhood of the pubes, and the adjoining skin of the groins and thighs. The buttocks were covered with a marked syphilide.

Typical syphilitic psoriasis was present on the palms of the hands and soles of the feet. Besides general swelling of the glands there were numerous plaques on the mucous membrane of the mouth and specific angina.

The patient, who apparently had taken no notice of the primary sore, must have become infected at the latest in April, 1905, since at the beginning of June he had developed secreting papules and headache. About 5 cc. of blood were withdrawn, with all precautions, from the median vein, care being taken to insert the needle through a perfectly normal piece of skin. A *Macacus rhesus* of medium size was then inoculated, a considerable amount of the blood being thoroughly rubbed into well scarified spots below both eyebrows and on the edge of the left eyelid. On October 31st, eighteen days after the inoculation, a slight brownish-red papule was just visible on the right upper eyelid. On November 2nd this had increased to the size of a small millet seed, was somewhat raised and covered with a non-scaly intact epithelium. On this day, the twentieth after inoculation, it was carefully sterilised and then scraped with a sharp spoon. The scrapings were spread over cover glasses and stained in the usual way with Giemsa's reagent after fixation in absolute alcohol. Numerous perfectly typical examples of the *S. pallida* with ten and more turns were found in the specimens. No other organisms were to be seen.

By this experiment the proof is given that the blood of a man, hitherto untreated, who had contracted syphilis at least six months previously, contained the *S. pallida* in so virulent a form that within twenty days after inoculation into a monkey a large number of typical spirochætes could be found in the primary lesion, although this had only been discernible for the preceding two days. On the other hand, according to the observations of the authors named above and according to my own experience, the incubation period following inoculation with material obtained from papules or primary sores is on the average somewhat longer.

The microscopic examination of the blood of syphilitic patients does not always lead to a positive result even when the method recommended by Noegerrath and Staehelin has been used. Hence the plan I have adopted might perhaps be of practical diagnostic importance in doubtful cases of syphilis, such for instance as in latent cases in women who have aborted, or in the mothers of children showing signs of congenital syphilis. It might also be useful from the scientific side in clearing up, for instance, the question of the so-called Colles' immunity.

I might add that I have also inoculated successfully two macaques and two long-tailed monkeys (*Cercopithecus callitrichus, Geoffr.*). The two long-tailed monkeys were inoculated in the ordinary way on the eyebrows, penis and skin of abdomen, the one with the tissue from a secreting papule, the other with the juice from an infected inguinal gland. In both cases a partially incrustated infiltration, which slowly extended peripherally, appeared on the eyebrows only. These healed after a few weeks. The macaques were inoculated with pieces of a recently affected inguinal gland. For this purpose the anterior chamber of the eye was opened with a sharp knife at the upper corneo-sclerotic junction, the iris torn through and the material introduced. In both, after about four weeks, a circumscribed infiltration appeared in the conjunctiva at the site of the wound. Shortly after the inoculation, iritis and keratitis developed, probably caused solely by the action of the foreign matter introduced. Five weeks after the first inoculation a second was made, this time under the skin. The material for these inoculations was from a highly virulent source (secreting papules with very numerous spirochætes), but in both cases gave no result, thus proving the positive character of the first inoculation. The determination of the question whether intraocular inoculation gives a negative result just like subcutaneous or intraperitoneal (Neisser and Metchnikoff) was thus prevented, since the virus had apparently caused a specific infiltration in the wound at the edge of the sclerotic. Hence the infection was quite analogous to a cutaneous one. None of the inoculated monkeys showed any general symptoms although they were under observation for three to four months. Three rabbits which I inoculated in the same way remained quite healthy, simply showing a local reaction which quickly disappeared. They were kept under observation for about four months.

FURTHER COMMUNICATION UPON SPIROCHÆTA PALLIDA, WITH DEMONSTRATION.

BY

E. HOFFMANN.

(Communication to the Berlin Dermatological Society on December 12, 1905.)

In a paper published a few weeks ago in the *Deutsche Medicinische Wochenschrift* (No. 42)[1] I have advanced the view I hold with regard to *Spirochæta pallida* and have stated, as the result of the examination of over 300 cases of syphilis in our Clinic, that it is probable, even that it is certain, that the spirochæte is to be regarded as the exciting cause of syphilis. Since then methods have been discovered by means of which it is possible, though not in every case, as I shall have to describe presently, to demonstrate the spirochæte within the tissues. This method is one of impregnation with silver. Bertarelli, Volpino, and Bovero in Turin were the first who succeeded in employing this beautiful method. Using a modification of van Ermengem's method of flagella staining they treated sections of a few μ thickness, and with it, as you will see in the original preparations of Bertarelli, have obtained most excellent results. Schaudinn and I have controlled the method, but while Schaudinn has succeeded in getting good results, I have failed. Frequently precipitates arise which may in some cases be removed by thorough washing with dilute gold-chloride solution.

A method by which it is possible to demonstrate the spirochætes in sections and which gives more certain results, is the modification of Ramón y Cajal's method for the staining of nerve fibrils worked out by Levaditi in the

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[1] See p. 44.

Pasteur Institute. Like most silver methods it appears to be very capricious. The publication by Levaditi upon the question and all the subsequent points which have been made out by him and his collaborators you will find in the *Comptes rendus de la Société de biologie.*

Bertarelli and Volpino stated in their paper that they had succeeded in finding the *S. pallida* in the liver and spleen of a seven months' syphilitic fœtus. I have seen one of Bertarelli's original preparations from the liver. Nearly every field contains an extraordinarily large number of the spirochætes, many more than one would have expected to find. Apparently in congenital syphilis at a certain period flooding of the tissues with spirochætes takes place.

Levaditi, partly alone and partly in conjunction with Salmon, has examined a large number of organs taken from cases of congenital syphilis. They have found the *S. pallida* in greater or smaller numbers in pemphigus bullæ, skin papules, liver, spleen, lung, kidney and suprarenals. Schaudinn and Paschen have also succeeded in demonstrating them in the placenta. By means of sections prepared according to Levaditi's method, Burnet and Vincent have found the spirochæte in a five days' old primary sore. In a moist anal papule, as you will see in the original preparation sent by Bertarelli, the spirochætes are to be found arranged in a very characteristic manner. They are present in the connective tissue of the papillæ, and especially abundantly in certain layers of the rete Malpighii, so that they have the appearance as if they were moving in swarms (like a haul of fish) towards the surface. We know that when these moist papules are mechanically irritated they secrete an abundant serum, which often contains large numbers of spirochætes. It seems to be a process by means of which a fresh infection of another person may be effected.

Among the preparations demonstrated are three which have been made by Schaudinn. One of these shows the *S. pallida* in large numbers in the alveolar epithelium of lung. A second shows them in smaller numbers in the epithelium of a kidney tubule, prepared from a child who had died from congenital syphilis soon after birth. The third shows spirochætes sparsely scattered through a placenta infected with syphilis.

The spirochætes lie in the connective tissue as well as in the cells of the lungs, liver, kidney, &c. They are especially found in the neighbourhood of the blood vessels, frequently also in the endothelium of the lymph and blood vessels, and even in the lumen of these vessels, where they may be seen at times within the lymphocytes. Quite recently Levaditi and Manouélian have found them within the blood vessels of a recently formed primary sore, and the spirochætes have already been found in the primary sores occurring in anthropomorphic and lower monkeys. It is only in cases of tertiary syphilis that no discovery of spirochætes has at present been made, even in the marginal areas of the tissues, where they may well be expected to occur, if they are to be seen at all in this stage of the disease.

With respect to my own investigations, as I have already noted, only a relatively small number of preparations have been successful in my hands. I have succeeded in finding the *S. pallida* in secreting genital papules, but only in scanty numbers. On the other hand, I have found the coarser form of spirochæte in the most superficial layers, as was indeed to be expected. This form only penetrates into the uppermost layers of the epithelium. One will have to bear this possible source of error in mind in the examination of sections, particularly those from secreting papules and ulcerating primary sores. In a few instances I have also seen them arranged in scattered clumps within indolent inguinal glands, but they were not perfectly typical. In phlebitis and lymphangitis syphilitica, and, as I may also add, in framboesia tropica, I have, when using this method, always failed to demonstrate them.

If now we conjoin the results obtained in these investigations upon the tissues to those previously obtained by the employment of the film method, there can no longer be any doubt that the *S. pallida* is, in fact, the actual exciting cause of syphilis. Naturally it remains most desirable that the final proof by the cultivation and inoculation of pure cultures of the spirochæte should be forthcoming.

I have selected a number of most beautiful microphotographs, prepared by Schaudinn, which show quite clearly the undulating membrane discovered by this author in the coarse variety and the long flagella of *S. pallida.* I may also mention that Schaudinn has seen an undulating membrane in the living *S. pallida*, but at present has not succeeded in staining or photographing it.

Finally, I would say a few words with regard to the diagnostic significance of the *S. pallida.* You are well aware that, unfortunately, the demonstration of the spirochæte entails much labour, and that not infrequently it is necessary to make several examinations before one succeeds. A number of conditions must be fulfilled, many of which are at present beyond our knowledge. Thus, one day one may find spirochætes in abundance, whereas the next none can be obtained from the same lesion. This may, perhaps, be explained in that the scraping with a sharp spoon has produced a partial stoppage of the lymph channels by clots. In a few days one may again find a few. In many cases, for some unknown reason, they may only be found on the third or fourth examination. The more carefully and accurately the examination is made, and the more practised one becomes at it, the fewer become the number of failures. The diagnostic significance is naturally of the greatest importance in the instance of quite recent primary sores, since the clinical diagnosis in these cases is often very uncertain. The finding of the spirochætes in these cases determines the diagnosis, while, on the other hand, a negative result from the reasons above given is of no special significance. Where it is a question of the distinction between the clinically similar soft sores, or herpetic erosions, the discovery of the spirochæte is, of course, decisive. In cases in which suspicion is aroused, solely from the presence of swollen glands, a positive result, obtained by the use of the method I have described for the purpose, will settle the diagnosis. In cases of latent syphilis the experimental inoculation of monkeys can serve as the final criterion. As I have shown, inoculation of monkeys with blood drawn from a patient may give positive results, though better are to be obtained by inoculation with the juice obtained by puncture of the lymphatic glands. In this way it appears to me that we possess means by the help of which we may usually settle the diagnosis of latent syphilis, a matter which has frequently proved insoluble.

Abstract of a Paper by THEODORE SHENNAN, *entitled :—*

SPIROCHÆTA PALLIDA (SPIRONEMA PALLIDUM) IN SYPHILIS.

(*Lancet*, 1906, *March* 10 *and* 17, *pp.* 663-667 *and* 746-752.)

[A SHORT account of the more important of the various organisms which have been described as the cause of syphilis is first given. These include bacteria of all kinds, cocci and bacilli. Several have believed that the causal agent is protozoal. Especial mention may be made of Siegel's[1 2] cytoryctes luis, because his work eventually led the Imperial Board of Health in Berlin to commission Schaudinn to investigate the whole question. A brief account of Schaudinn and Hoffmann's papers is next given, and this is succeeded by references to the researches which have been evoked as a result of their discoveries. These abstracts are given verbatim in the following pages, omitting those which have been already referred to in the preceding or succeeding papers of this volume.]

Raubitschek[3] and Frosch[4] detected the *Spirochæta pallida* in the circulating blood in secondary syphilis.

Wechselmann and Löwenthal,[5] using the "ultra-microscope," described the spirochæta as made up of several segments and noticed what they took to be a nucleus.

Salmon[6] confirms this result in the pemphigus of congenital syphilis.

Jacquet and Sevin[7] found *S. pallida* in secondary lesions, but failed in twenty-three tertiary lesions examined.

Kraus[8] insists on the necessity for making a considerable number of preparations, as the spirochætæ are often very irregularly and unequally distributed. He observed that in a chancre kept twelve hours on ice, no spirochætæ could be demonstrated, although they were visible in fresh preparations. He has never found *S. pallida* in controls.

Volk,[9] in the chancres and papules in 14 syphilitics, found the parasite in the great majority. The results were positive in only 1 out of 14 syphilitic glands, and were negative in the glands connected with 17 control, non-syphilitic lesions.

Horand[10] refers to his published papers describing a "hémoprotiste, agent pathogène de la syphilis," and believes that Schaudinn's parasite corresponds to one of the involution forms of this "hémoprotiste."

M. Schüller,[11] in a review of Schaudinn and Hoffmann's paper on the Occurrence of *S. pallida* in Indolent Buboes, doubts whether there is any etiological relationship at all. The parasite may have entered through the ulcerated surfaces. Moreover, bacteria have been found in glands in connection with non-ulcerated lesions and also in congenital syphilitic lesions. He thinks that the presence of *S. pallida* in experimental lesions in monkeys does not clear up the question, but simply shows that along with the cause of syphilis the spirochætæ and any other bacteria present have been transferred. He also criticises the technique employed and cannot understand Schaudinn's grounds for placing spirochætæ amongst the animal parasites.

Vuillemin proposes the name "spironema" for spirochætæ with sharp ends. The *S. pallida* would thus become the *Spironema pallidum.*

McWeeney[12] found *S. pallida* in nine primary and secondary cases, not in a tertiary ulcer of the palate or in a non-ulcerated, muco-purulent vaginitis. He thinks it possible that tertiary lesions and congenital forms of syphilis may be due to a chronic intoxication caused by absorption of metabolic products of the spirochætæ.

C. Fraenkel[13] writes enthusiastically in support of Schaudinn and Hoffmann, and goes further than these authors, claiming that there is no doubt that *S. pallida* is the cause of syphilis. He found that organism in six varied cases of syphilis.

Vilh. Jensen[14] found *S. pallida* in a hard chancre and the associated gland, and in several entire and ulcerated papules in the region of the genitals. They were not found in control non-syphilitic cases. He believes that the true cause of syphilis has been found.

Bordet and Bayet[15] consider this the most serious attempt yet made to unravel the etiology of syphilis. Bayet found *S. pallida* in the spleen of a congenitally syphilitic infant. Lassar[16] discusses the discoveries of Siegel and Schaudinn and reminds his readers that during the last three decades more than twenty-five causes of syphilis have been described.

K. Herxheimer and H. Hübner[17] demonstrated spirochætæ with Giemsa's stain and also with Nilblau B.R. or Capriblau. They examined 18 cases and in 15 of these they found *S. pallida*, but only in extremely small numbers in some cases. In two of the remaining cases the diagnosis was between soft sore and syphilis. In these no *S. pallidæ* were found and the subsequent course of the illness justified that result. They claim to have seen a spirochæta in a section cut in paraffin and stained with Nilblau. Examinations of blood, lymph glands and congenital syphilitics proved negative.

Kiolomenoglou and von Cube[18] confirmed the fact of the occurrence of *S. pallida* in syphilitic lesions, but they considered it still more important to ascertain whether it was always absent from non-syphilitic lesions. They exercised the greatest care in making and staining their preparations. They found *S. pallida* in a collection of syphilitic cases, in an inflamed phimosis in which there may have been a masked primary sore, in gonorrhœal pus from Bartholini's gland, in balanitis, in pus from a scrofulo-dermatic abscess, in an ulcerating cancer, and in condyloma acuminatum. They found forms intermediate between *S. pallida* and *S. refringens* and corresponding to neither. Schaudinn, to whom their preparations were submitted, confirmed their opinion that there were more than two species of spirochætæ present, but, with Hoffmann, declares that all occurring in non-syphilitic lesions can be, either morphologically or tinctorially, distinguished from *S. pallida.* Still, one feels that they do not conclusively dispose of all the objections of Kiolomenoglou and von Cube.

Hoffmann,[19] with Mulzer, found spirochætæ in ulcerated carcinomas of the cervix and skin very similar to *S. pallida*, and which they could not with confidence distinguish from it. In a later paper[20] Hoffmann states that those found in carcinomata have blunt ends.

Babes and Panea[21] [22] found *S. pallidæ* in two out of three cases of congenital syphilis, in one case occurring especially abundantly in the suprarenals. They were found where characteristic histological changes were best seen, *e.g.*, the liver; but in the suprarenal such changes were not so marked, though the organisms were in great numbers.

Galli-Valerio and Lassueur[23] emphasise the fact that *S. pallida* has been found in closed lesions, *e.g.*, indolent buboes. They detected it in condylomata and in mucous plaques in six out of ten syphilitics. They failed in two cases undergoing treatment and in a tertiary case, also in a hard chancre and gland connected therewith, and in the cerebro-spinal fluid of two cases.

Krzysztalowicz and Siedlecki (Krakow)[24] give an interesting historical retrospect of the search for the cause of syphilis, and furnish full references. They describe several delicate shapes in the fluid from excised glands, very motile, at first oval, but on longer observation elongating at both ends, as if sending out processes in one line. They describe apparent longitudinal splitting up of spirochætæ similar to what is seen in trypanosomes. They figure coarse spirochætæ as pallidæ, which one should rather incline to place under the "refringens" class. They state that the numbers of *S. pallidæ* increase in proportion to the distance from the surface.

Thesing, who at the first discussion in the Berlin Medical Society suggested that the spirochætæ came from the staining fluid, believes[25] [26] that they are harmless saprophytes occurring secondarily in syphilitic lesions and penetrating to glands from the surface. He thinks, moreover, that such a flexible body as *S. pallida* should not be tied down to the constant possession of steep spirals.

Ploeger[27] discusses the morphology and development of spirochætæ and distinguishes them from spirilla by the flagella, which the latter possess. He suggests that all spirochætæ the spirals of which measure about 1 μ or less should be placed with *S. pallida*, and those with more than 2 μ with *S. refringens*. He describes short forms with a terminal knob. He found the *S. pallida* in two chancres, one gland, and in three anal and one vulvar papule. One cannot be certain what proportion this was of the cases examined. Some of the forms seen in films from glands were 20μ long. He, as well as Rille[28] and Mulzer,[29] has seen some apparently attached to red blood corpuscles, and the suggestion is made that these cells may convey the parasite throughout the body.

Spitzer[30] demonstrated the presence of the *S. pallida* in six primary sores; in seven secondary cases even after prolonged treatment; in one ulcerating case of eight years' duration (Spätform), with infiltration of the nose, lips and lower lid; and in a case twelve years after infection in a gumma of the scalp. In this case only one spirochæta was found in three or four preparations. He never found spirochætæ in the blood or in a series of non-syphilitic conditions.

Rille[31] refers to the behaviour of the *S. pallida* in physiological salt solution in glycerine. He refers to Bordet's and Gengou's observation of these spirochætæ in 1902, and says that he himself saw them in 1894. He had lately positive results in three primary lesions in the male, in one in the female, and once in an inguinal bubo and condyloma latum. He thinks that the possibility of the *S. pallida* being a morphological variant of the *S. refringens* should be excluded. He refers to Donné's discovery of "vibrio lineola" (1837) and his belief that it was the cause of syphilis.

Noeggerath and Staehelin[32] found the *S. pallida* in syphilitic blood by the following method. They take one cubic centimetre of blood from a vein, mix with ten cubic centimetres of one-third per cent. acetic acid in water, centrifugalise, and examine the deposit.

Neumann[33] found the *S. pallida* in six cases, two of which had been treated.

Queyrat and Joltrain[34] found the *S. pallida* in a quarter of their cases (31 in all) of hard chancre, but never in non-syphilitic lesions. They think that it must have a diagnostic signification.

Jesionek,[35] discussing Ploeger's paper,[27] refers to the conclusions of Kiolomenoglou and von Cube and maintains that their results are correct. He refers also to the intermediate forms and suggests that *S. pallida* and *refringens* may be developmental stages of one and the same organism. This was before spirochætæ were found in the blood.

R. Kraus and Prantschoff[36] contribute an important paper. They find that spirochætæ disappear from excised chancres when kept, and quote Metchnikoff and Roux, who found that after six hours an excised lesion was useless for the demonstration of spirochætæ. This is interesting in view of the generally accepted clinical fact that the syphilitic virus removed from the living body or after death soon degenerates. They found the *S. pallida* in 32 out of 37 hard chancres ; in the negative cases the lesions were necrotic, purulent, or the material was delayed too long in transit. Eighteen out of twenty-five papules gave positive results. In the lesions in four macacus monkeys *S. pallidæ* were found. Like Ploeger, they saw short forms with from three to four spirals, with spherical bodies either attached to one end or free. They suppose that they are involution forms analogous to those seen in Pfeiffer's phenomenon in the case of the cholera vibrio. On making sections of an excised sore parallel to the surface and examining films from each, they found that true *S. pallidæ* increased in numbers as they went deeper.

Levaditi and Petresco[37] find that the spirochætæ pass readily and in considerable numbers into blisters produced by cantharides on the surface of syphilitic lesions. They have been successful in this way in demonstrating *S. pallidæ* in roseolar eruptions.

Bandler[38] details the methods of staining he has found successful.

Leiner[39] found *S. pallida* in congenital syphilitic pemphigus. Lannois[40] saw Schaudinn's spirochætæ associated with Vincent's bacillus fusiformis. Almkvist and Jundell[41] found the spirochætæ six times in seven cases, but never succeeded in films from glands, blood or non-syphilitic lesions.

Mulzer[42] demonstrated *S. pallida* in films from 20 out of 22 cases. One of the failures was from a roseolar eruption. He occasionally found them in cells, probably of endothelial nature. Some showed a spindle-shaped swelling between the middle and terminal thirds. In one case he found a mesh-like agglomeration of from 20 to 40 individuals. No *S. pallidæ* were found in normal smegma in five women and ten men. He quotes Csillag's negative results in three men and 16 women. Rona found spirochætæ in six out of 20 healthy women and in three out of 18 healthy men. Menge-Krönig found them also in women. In 21 out of 29 patients with non-specific ailments Mulzer found no spirochætæ.

Nigris[43 44] claims that he found the two varieties together in the blood. Hoffmann,[47] who saw the preparations, denies that this is the case.

Scholtz[45] doubts whether *S. pallida*, even when occurring in great numbers in syphilitic lesions, has an etiological significance. It may be that the conditions produced are specially favourable for that organism to grow and develop. He found an apparently undoubted *S. pallida* in a condyloma acuminatum. In primary and secondary lesions he had 19 positive and 11 negative results, and two positive and one negative result in congenital syphilis. He failed to find spirochætæ in roseolar blood in two cases, in buboes in two cases, and in tertiary syphilis in four cases. In 12 controls the results were negative. Hoffmann[47] examined the film from the condyloma acuminatum and averred that only the *S. refringens* was present.

Grouven and Fabry[46] had positive results in 14 out of 20 primary and secondary lesions, in two cases of congenital syphilis, and in syphilitic blood, following the method of Noeggerath and Staehelin.[32]

Rille and Vockerodt[48] in 14 syphilitics found manifest *S. pallidæ* in 22 different lesions. They failed in roseolar blood and syphilis hæmorrhagica neonatorum. With Ploeger they think that the spirochætæ have a relationship to the red blood corpuscles. They found them in a case seven or eight years old.

Sobernheim and Tomasczewski[49] in 50 primary and secondary syphilitics found *S. pallida* in all cases. In eight tertiary syphilitics they failed. In some of the positive results spirochætæ were very scanty and found only after prolonged search. They doubt whether they are actively motile. They consider that *S. pallida* is the cause of syphilis.

Siebert[50] has found spirochætæ which agree strictly with the type "pallida" indicated by Schaudinn and Hoffmann only in syphilitic lesions. He refers to the difficulties of distinguishing them morphologically, and points out that there is no analogous disease in which the number of the causative organism bears no relation to the intensity of the reactions. Here the organisms are generally scanty. He found spirochætæ which in delicacy could not be distinguished from the *S. pallida*. He is evidently not wholly convinced. His results are positive in 52 out of 66 cases of primary, secondary, and congenital syphilis; negative in six indolent buboes, in seven tertiary cases, in blood and spinal fluid. He confirms the observations of Klingmüller, Baermann, and Metchnikoff, that the syphilitic virus cannot pass through a porcelain filter. His results were also negative in 46 control non-syphilitic cases.

Flügel[51] examined 28 cases, in all being successful. Some showed only one *S. pallida* after very prolonged search. In the liver of a congenitally syphilitic child he found only one spirochæta. They were frequently in relation to red blood corpuscles (*cf.* Ploeger, Rille, Scholtz, and Roscher). He found them in the blood by the method of Noeggerath and Staehelin.

Brönnum and Ellermann[52] found *S. pallida* in two cases of congenital syphilis.

Roscher[53] produces a very long, complete, and carefully detailed paper in three parts. Of primary affections, genital and extra-genital, 31 gave positive results and one was negative. In 16 there were no general evidences of syphilis, but in 11 of these which continued under observation secondary signs appeared later. Thirty buboes gave positive results and eight were negative. Fifty-five moist papules were positive, three were negative, of which one had been treated, one was dry, and one was not scraped deeply enough. Thirty-four non-ulcerated papules gave positive results, and six were negative. Plaques on the tonsils were 14 times positive, once negative. Two plaques on the tongue and 14 papules on the lips or at the angles of the mouth were positive. In these coarse spirochætæ, in addition, were regularly found. Impetigo of the scalp gave four positive results, and blood three positive and three negative results. He never found coarse spirochætæ in glands or in closed efflorescences. In all, 206 syphilitic lesions were examined, 184 with positive and 22 with negative result.

De Souza, jun., and Pereira[54] found *S. pallida* in nine primary and secondary cases, and in one congenitally syphilitic infant with pemphigus. Cultures in human blood with 5 per cent. each of sodium citrate and sodium chloride were negative.

Reischauer[55] found *S. pallida* in the liver, spleen and lungs of a congenitally syphilitic infant.

Hoffmann succeeded in infecting four monkeys (macacus Rhesus, two, and cercopithecus, two) with syphilitic blood.

Babes and Panea[21] found *S. pallidæ* in great numbers in the adrenals and liver of congenital syphilis. They suggest that they are like bacterial flagella and might be demonstrated by similar staining methods. They have found spermatozoa-like bodies in the blood of congenital syphilis.

Oppenheim and Sachs[56 57] succeeded in finding *S. pallida* in 39 hard chancres and papules, failing in 21. They also failed in nine buboes, 21 examinations of blood, 15 roseolar spots, two mucous plaques, seven gummata, and four cases of congenital syphilis. In 42 non-syphilitic conditions they found no *S. pallidæ*.

Bertarelli and Volpino[58] found *S. pallida* in 26 out of 42 primary and secondary cases. By the use of a silver solution method they demonstrated great numbers of spirochætæ in *sections* of the liver of a congenitally syphilitic child, and by control observations showed that they had not to do with elastic fibres, connective tissue fibrils or nerve-endings. Cultures of syphilitic blood were all negative.

Herxheimer and Löser[59] describe the presence of granules in the *S. pallida*, possibly of the nature of blepharoblasts, also small free bodies with nucleus, protoplasm and membrane, possibly representing a developmental stage. They think that appearances formerly described as vibratile membrane were simply artefacts. They confirm the presence of flagella.

Nicolas, Favre and André contribute an important paper.[60] They describe the staining methods which they find most suitable. Their positive results were four out of 16 hard chancres, eight out of 14 mucous plaques, one out of seven buboes, *but not pure.* Examination of two cases of syphilitic pemphigus and the blood in three cases was negative. They examined numerous non-syphilitic lesions without finding the *S. pallida.* They indicate certain changes of aspect which this parasite may undergo in course of preparation. They state that Widal and Ravant have examined the cerebro-spinal fluid of 15 syphilitics without finding *S. pallida.* They are not altogether convinced that spirochætæ found in some other non-syphilitic lesions are not *S. pallidæ.*

Burnet[61] refers to the finding of spirillar forms in spirillosis of birds, by Sacharoff in the Caucasus and by Marchoux and Salimbeni (1902) in Rio de Janiero ; in sheep by Laveran and Theiler in the Transvaal (1903) ; and in tick fever in man by Dutton (1905). He draws analogies between dourine (mal de Caděras, maladie du coït) and syphilis, the former being due to a trypanosome which can penetrate a healthy mucosa—*e.g.*, vulva of a dog or rabbit. He has seen Schaudinn's photographs of the flagella of *S. pallida* and is not convinced of the reality of the flagella. He agrees with Vuillemin and Schaudinn that the *S. pallida* is not a true spirochætæ and is better described under the new name of *Spironema pallidum.* He describes ten different methods of staining.

Bandi and Simonelli[62 63] found *S. pallida* in the blood from a roseolar spot and in three out of five secondary lesions.

Fanoni[64] gives his results with the original and new Giemsa and with Marino's blue. Three photographs of *S. pallida* illustrate the article, of which No. 2 (×1,400) is good, but Nos. 1 and 3 are very doubtful, particularly No. 3, which is apparently either a wavy piece of fibrin or a drawn-out nucleus.

Gordon[65] failed to detect *S. pallida* in the lumbar puncture fluid from cases of cerebro-spinal syphilis or tabes with a distinctly syphilitic history.

Paltauf[66] discusses the question of the bacterial or protozoal nature of *S. pallida.*

Lipschütz[67] discusses the whole question very ably, very carefully, and in detail. His paper merits close study. His conclusions, drawn from a survey of the literature, are distinctly good. He notes that the age of the lesion and even a short energetic course of mercury seem to have little influence on the demonstration of *S. pallida.* Others[68] state that they disappear during mercurial treatment. In 33 out of 49 cases of primary and secondary syphilis he demonstrated *S. pallidæ.* In three typical gummata the results were negative. In 12 non-syphilitic controls the results were negative.

K. Herxheimer[69] in a later paper gives his method of staining with a hot saturated solution of gentian violet after alcohol fixation. He describes the larger spirochætæ as having a double contour, best seen in photographs taken against a dark background. He also describes three forms of granules which he thinks may have something to do with the developmental stages of the parasite—one group within the spirochæta, another attached to it, and the third group free. Little can be said with regard to these except to record the observation. He refers to rodlets and sausage-shaped bodies described by Wechselmann and Löwenthal.

Richards and Hunt[70] describe three varieties of spirochætæ found in syphilitic lesions, one thick, straight or slightly curved, another thick but spiral, and the third delicate and spiral, corresponding to *S. pallida*. They found the last organism in three cases in blood from roseola, and in one of these cases in films taken on each of ten successive days.

Dalous[71] refers to the history of the search for the cause of syphilis. He states that by the use of quartz lenses, with a magnification of from 2,500 to 4,000 diameters, an undulating membrane can be detected.

Bandi and Simonelli detail several methods of staining *S. pallida*, and describe a simple and rapid method they have elaborated.[72] It somewhat resembles Jenner's method.

Pollio and Fontana[73] found *S. pallida* in eight out of 19 cases of primary and secondary syphilis. They failed altogether in tertiary syphilis, in other venereal ulcers, herpes progenitalis, acne vulgaris, folliculitis and aphthous stomatitis.

Risso and Cipollina[74] give details as to nine cases of primary and secondary syphilis. They were successful in demonstrating *S. pallidæ* in four out of five buboes and in two mucous plaques, where they were few in numbers. They failed in films from circulating blood, five times; blood from spleen, once; primary sore, twice; and papule, once. One of the hard chancres was cicatrising, and in the other several injections of mercury had been given. Examination of two gummata was negative.

Pascalis[75] concludes that *S. pallida* is demonstrable in all primary and secondary syphilitic lesions and never in non-syphilitic lesions.

Hubner[76] refers to the history of the discovery and reviews the literature. The paper is interesting as indicating the countries to which the different writers belong and the dates on which they made their discoveries and communications; also, and what is more important, he assigns to each man his share in adding to the knowledge of the subject. He thinks that the tertiary cases in which Rille found *S. pallida* were more likely very late secondary cases, and that Vincent's bacillus fusiformis may be a developmental form of a spirochæta. He concludes that syphilis is a chronic spirillosis which is produced by a spirochæta pathogenic not only for man but also for the old-world monkeys. He attempts to draw analogies between syphilis and recurrent fever, which is also a microbial disease pathogenic for man and also for monkeys. He compares the irido-choroiditis which may occur in this fever with that occurring in syphilis. Of course, the analogy breaks down when one considers, firstly, the chronicity of syphilis and the acuteness of recurrent fever, and, secondly, the mononuclear leucocytosis of syphilis and the polynuclear leucocytosis of recurrent fever.

Veillon and Girard[77] by Levaditi's method found spirochætæ in the roseolar rash. They suggest that the congestion is not toxæmic but the result of a veritable embolism of the terminal capillaries of the papillæ of the corium.

Moncorvo fils[78] has found spirochætæ in congenital syphilis. He uses hydro-alcoholic solutions of saffranin and violet de dahlia.

Bodin[79] in 37 cases found *S. pallida* 21 times. He concludes that it is present constantly in untreated developing chancres ; in secondary syphilides—papular, papulo-squamous and ulcerated ; and in ano-genital syphilides (condyloma). The organism disappears rapidly under the influence of mercurial treatment, whether general or local, and with local application of antiseptics (all observers do not agree on these points). He has never found *S. pallida* in tertiary lesions. He finds the spirochætæ in constant relation to red blood corpuscles. In conclusion, he thinks that their occurrence so constantly in the lesions cannot be considered merely a coincidence.

Buschke and Fischer have demonstrated clearly the spirochætæ in sections by silver impregnation methods. This was first done by Bertarelli and Volpino.[58] Levaditi modified the procedure according to the method of Ramon y Cajal and got similar results, not only in the organs of a congenitally syphilitic infant but also in primary sores and secondary papules of an adult, and, in addition, in a primary sore in a monkey. Paschen demonstrated them by the same method, and Buschke and Fischer also adopted it. The details of the method are given. Their successes included a condyloma latum, the liver and spleen in two congenital syphilitics, and the kidneys and cutaneous papules in another. The examination of the liver from a man with tertiary syphilis proved negative.

Taylor and Ballenger[80] found *S. pallidæ* in two hard chancres and one mucous plaque. Neisser and Baermann[81] had 27 positive and 12 negative results in man. In four out of 12 monkeys they had positive results.

"Gathering together the details from this not quite complete survey of the literature on the subject, and from my own experience, I may now piece together an account of the character of these spirochætæ as at present understood. The names *S. pallida*, or *Spironema pallidum*, and *S. refringens* are descriptive, the former staining faintly, the latter being markedly refractile in the living state. The *S. pallida* is an extremely delicate organism, weakly refractile when living, vigorously motile, stained with considerable difficulty, and difficult to demonstrate, requiring at least a magnification of 800, with good illumination. It is long, extremely thin and filamentous, of a distinct spiral or corkscrew shape, both when living and when fixed on the slide. It tapers at the ends to a sharp point. In length it varies from 4 to 20 μ; in breadth it measures about 0·25 μ. The spirals number from 6 to 26, averaging from 8 to 10. Not only are these spirals numerous, but very narrow—not over about 1 μ—regular and deep. Schaudinn, Hoffmann, Herxheimer, and others describe terminal flagella, but all observers are not agreed upon this point. When these are double they may really represent the commencement of a longitudinal fission. The spirochætæ may lie in clumps and occasionally form a felted mass. Löwenthal thinks that they are made up of several shorter individuals (from 3 to 4 μ), or possibly these may be degenerative forms. With Herxheimer, Mulzer, and others, he describes a granule or localised swelling between the middle and one terminal third, which may represent a blepharoblast rather than a nucleus. Some describe a terminal globular body, the real nature of which has not yet been ascertained. It may simply be a loop at the end of the spirochæta. Herxheimer has described other bodies in, on, or near the spirochæta. At first it was thought to possess a vibratile membrane attached throughout its whole length, but this idea is given up by most authors, with the exception of Dalous, the appearance being put down as an artefact. The absence of such membrane along with the possession of flagella are held to be characters distinguishing it from other spirochætæ which commonly occur on ulcerated surfaces. Its motility is of three kinds: corkscrew, to and fro, and bending or lashing. In a film dried on the surface of glass the spirals may become stretched out owing to its becoming attached first at its extremities. By silver impregnation methods it has been demonstrated in sections of the liver in congenital syphilis and in the primary and secondary stages of the acquired disease. It lies in the capillaries of the papillæ of the corium, chiefly lying up against the endothelium or in the lumen. Thence it makes its way into the surrounding tissues and works up between the cells of the Malpighian layer, to reach but not to penetrate the stratum corneum. With Giemsa's stain it takes a rose-pink colour, or slightly lilac if the staining be too prolonged. It may also be demonstrated by 'flagella' stains and impregnation methods, by gentian violet, carbol fuschin, cresyl violet, Nile blue, &c. Schaudinn places *S. pallida* with the protozoa rather than with spirillar bacteria, allying it to the trypanosome group.

"The *S. refringens* is generally acknowledged to be of saprophytic nature, and corresponds to the forms already described in normal smegma, in balanoposthitis, vulvovaginitis, on the surface of ulcers about the genitals, on venereal warts, in the mouth secretions, on the teeth, tonsils, in ulcerated carcinomas, and probably also the forms found in gangrene are closely related. It is thicker than *S. pallida*, distinctly refractile. It may have a marked corkscrew shape and motion when alive but flattens out when dried in films, so as to have usually a wavy outline. The curves are flatter and longer than those of *S. pallida*, measuring from 1·5 to 2 μ or more. It varies in length like the *S. pallida*, measuring most commonly from 8 to 10 μ. It is usually described as having truncated ends, but often it has tapering ends, or one end is sharp and the other blunt. It possesses a vibratile membrane but no flagella. It stains easily, quickly, and deeply by all methods, and with Giemsa's stain, which is the only differential one as yet known, it takes a purplish or violet colour. It occurs usually only on the surface of moist and ulcerated syphilitic lesions. Some describe it as invading the glands.

"*Demonstration of these Parasites.*—From the literature I have picked out the details as to 758 lesions examined by different workers, and find that *S. pallida* has been found most often in moist papules, mucous plaques, and condylomata, genital and extra-genital — 159 positive and 26 negative. Next come hard chancres, excised or scraped—150 positive and 55 negative; non-ulcerated papules—92 positive and 31 negative. Success has not been as frequent in films made from excised or punctured buboes—52 positive and 30 negative, and rarely has *S. pallida* been found in roseolar blood, four positive and 17 negative—or in the circulating blood, five positive and 37 negative.

"In lesions with moist or ulcerating surfaces the *S. pallida* is commonly accompanied by the *S. refringens*, and it is a very significant fact that most successes have been attained in such circumstances. From one's own experience the number of forms intermediate between the two, partaking to some extent now of the one, now of the other, is remarkable. It is sometimes extremely difficult, often impossible, to refer these forms to the one group or the other, and it is easy to imagine how readily one's percentage of successful demonstrations of the *S. pallida* in such lesions might be raised by having regard solely to tinctorial or morphological characters."

Dr. Shennan next gives an account of the 21 cases he had himself examined.

Of these 21 cases, two (8 and 12) were not syphilitic; another (21) was a case of tabes dorsalis three years after infection. Excluding these there are left 18 cases with

25 lesions examined. Out of the 18 cases six showed the presence of undoubted *S. pallida.* In three others spirochætæ were found which could not with certainty be placed either with *S. pallida* or *S. refringens.* One case (7) was examined when I had little experience in looking for *S. pallida.* In one case (9) the possibilities are in favour of the bodies seen being *S. pallidæ.* In two cases (14 and 15) the lesions were drying up under treatment. The results were quite negative in the remaining five cases.

As regards the lesions, five hard chancres showed positive results; in two the results were doubtful and in three they were negative. Two non-ulcerated papules gave positive results; two dry papulo-squamous syphilides gave negative results, and two roseolar rashes also were negative. Four condylomata were examined; three were negative and one was doubtful. Four glands were examined and in none could undoubted *S. pallidæ* be found. One rupoid syphilide was examined with a negative result. Typical *S. pallidæ* were found only in hard chancres and in closed papules—*i.e.*, in typical syphilitic lesions.

CONCLUSIONS.

"It is astonishing with what unanimity this discovery has been accepted on the continent, an occurence quite unprecedented in discoveries of similar importance, but there is much to support the relationship of *S. pallida* to syphilis. The most important facts in favour are its occurrence in non-ulcerated lesions, such as papules and buboes far away from the primary sore; its occurrence in the circulating blood and splenic blood in acquired syphilis; its occurrence specially in virulent infective lesions, and its absence from non-infective tertiary lesions; its presence in the depth of superficial lesions where it is stated to be unaccompanied by other organisms, and its definite localisation in the corium —if absolutely confirmed; its presence in congenitally syphilitic infants in great numbers, and its occurrence—according to most observers—only in primary and secondary syphilitic lesions and in congenital syphilis—never in non-syphilitic diseases. Against it is its frequently scanty numbers; films in very many of the reported successful cases revealing the presence of only one or two spirochætæ after repeated and prolonged search, the numbers thus frequently bearing no relationship to the severity of the tissue reactive changes, which is, as Siebert points out, without analogy in the domain of pathology; the possibility, which is by no means disproved, that various organisms may invade glands, penetrating from a superficial ulcer; the difficulty of diagnosis in the case of the numerous intermediate forms which are found so commonly in such highly infective lesions as the mucous plaque, &c.; the claim, still strongly upheld by some, that forms indistinguishable from *S. pallida* are found in non-specific lesions. The last point, of course, even though it were granted, does not necessarily invalidate a causative relationship to syphilis.

"In conclusion, one may say that while there is a very considerable probability that *S. pallida* bears an etiological relationship to syphilis, we require to know more about spirochætæ in general and to have better methods of distinguishing the many forms intermediate between the typical *S. pallida* and the typical *S. refringens*, before we can unreservedly accept this as a completely proved fact in pathology. One can say very little as yet with regard to the peculiar loop-like and rounded bodies found in syphilitic glands, and to such bodies as the flagellated structure I have described in a film from a hard chancre."

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ON THE PRESENCE OF SPIROCHÆTES IN YAWS.

BY

ALDO CASTELLANI, M.D.

(Abstract.)

In a paper read before the section of Tropical Diseases at the Annual Meeting of the British Medical Association at Leicester (1905), Dr. Castellani announced his discovery of the presence of spirochætes in yaws.[1] In February, 1905, whilst examining films from the ulcers in a case of parangi, stained by the Leishman-Romanowsky method, he noticed several minute, almost invisible, spirochæte-like bodies. On reading Professor Schaudinn's preliminary note he proceeded to examine cases of yaws more closely. In two who were suffering from dry lesions, no spirochætes were found; in two others they were found to be present. One was examined once only. The other was examined several times. He was a Singalese boy, aged 10, with no signs of acquired or congenital syphilis. Spirochætes were found both in the surface of the lesions, and also in deeper scrapings. In hanging drop preparations they could be distinguished easily by their characteristic movements. He sent films from this case to Professor Schaudinn, who found them to contain three varieties of spirochætes, one of which, very delicate, resembled very closely the *Spirochæta pallida*.

In two other communications to the *British Medical Journal*, Castellani recorded the results of further observations.[23]

"In the preparations taken from ulcerated lesions, according to Schaudinn and myself, various spirochætes are present. One form is rather thick and takes up the stain readily; it is morphologically identical with the *S. refringens* of Schaudinn. One form is thin, delicate, with waves varying in size and number and with blunt extremities; I proposed for this variety the name of *S. tenuis obtusa.* A third form is also thin and delicate, but is tapering at both ends. I named it *S. tenuis acuminata.*

"In non-ulcerated lesions there may be found a spirochæte which I believe to be identical with the *S. pallida.* The organism is extremely delicate, thin, and generally tapering at both ends. The length varies from a few microns to eighteen to twenty microns. The number of waves varies also, but they are generally numerous, uniform and of small dimensions. Sometimes two spirochætes may be seen attached together, or apparently twisted one on the other. Two organisms close together and nearly parallel, but united at one end, as described in *S. pallida* by Schaudinn and McWeeny, have been seen."

Professor Schaudinn also examined the films from the above-mentioned non-ulcerated lesions and found that they contained spirochætes morphologically identical with *S. pallida.*

Castellani noticed that patients with yaws did not present the spirochætes constantly but at intervals. He did not find the organisms in any lesions which were in the healing stage.

Summarising his results Castellani found spirochætes in eight out of eleven cases of yaws[2 3]; and among the spirochætes there was a variety morphologically identical with the *S. pallida* of Schaudinn.

The above recorded discovery of spirochætes in yaws affords strong confirmation of the correctness of Mr. Jonathan Hutchinson's attitude in maintaining for years his view of the common origin of syphilis and yaws. The following extract is taken from the conclusion of his exhaustive survey of the question in the "Atlas of Clinical Medicine."[4]

"To my mind it seems most probable that the virus—as yet undemonstrated—which causes these affections is one and the same, and that it has had its homes, from times long antecedent to medical history, in various parts of the world. Amongst these were the west coast of Africa, the West Indian Islands, Fiji and other islands in the Pacific, China, and possibly India. It was not known in Europe until the end of the fifteenth century, when it was introduced by Portuguese traders from West Africa. Almost simultaneously, but a little later, it was also brought over by Columbus' sailors from the West Indies. It then spread over Europe as 'the Neapolitan Disease,' the 'French Disease,' and finally, as 'Syphilis.' With the progress of knowledge and increase of care it became more and more restrictedly a venereal malady, but in the first instance it was not so, and is still not so exclusively. From the first it occasionally produced, in its secondary stage, a frambœsial eruption, and to cases of this type the names of 'button scurvy,' 'sibbens,' 'morula,' and some others, were, in different places, applied. In tropical countries and amongst coloured races the frambœsial type of eruption is much more frequent than in Europe or North America. In tropical countries also, syphilis spreads very commonly as a non-venereal disease, the primary sore occurring not on the genitals, but on some part of the trunk or limbs. Flies are probably very frequently the carriers of the virus; the naked skin, when abraded by accident, offering ready sites for contagion. The subjects of these non-venereal cases are often children, and the primary sore is usually ill characterised and is often overlooked. Hence the confusion which has arisen.

"No one would ever have thought of differing from the opinion of Sydenham and his contemporaries had it not been for two circumstances: first, that in so-called yaws the primary sore is usually not on the genitals and is therefore often overlooked; and second, that the secondary eruption is usually frambœsial. It is the frambœsial eruption which makes such a strong impression upon the minds of observers. But this eruption is really only a transitory stage, and in all other features, both antecedent and subsequent, the disease corresponds with European syphilis. In the latter disease we still see occasionally cases of the most marked frambœsial type. It is, then, simply in the frequency of this type of eruption that the two maladies differ, and this frequency may be reasonably attributed to difference in climate and race."

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FOUR COMMUNICATIONS

GIVING THE RESULTS OF

Experimental Investigations on Syphilis

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EXPERIMENTAL INVESTIGATIONS ON SYPHILIS.

BY

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First Communication.

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HITHERTO the actual cause of syphilis, that great scourge of humanity, has remained unknown, and the whole subject of its pathogeny and of immunity in relation to it has been confused and uncertain.

In former times, when it was believed that the disease could be easily and completely cured, and when its serious late manifestations were unknown, inoculations were made on human subjects in order to clear up disputed points. But with our present knowledge of the far-reaching after-results of syphilis such inoculations are unjustifiable, and search has been made for animals susceptible to the syphilitic virus.

Many attempts have been made to inoculate various classes of vertebrata :—cold-blooded animals, such as frogs and salamanders (Brieger and Uhlenhut); different kinds of birds; and, still more frequently, mammals. In the great majority of instances the results have been negative. But from time to time it has been stated that the animals experimented on have developed more or less definite syphilitic lesions. Thus Auzias-Turenne[1] claimed to have obtained syphilitic papules and mucous patches in a cat as far back as 1866; and in the following year Legros and Lancereaux[2] described syphilitic lesions in a guinea-pig which had been inoculated with a portion of a hard chancre. The pig, among other mammals, has frequently been the subject of inoculations. So far as we know, Martineau[3] was the first to claim that this animal was susceptible. After inoculating a young pig with pus from a chancre he observed a parchment-like induration at the site of inoculation, followed by the appearance of numerous papules on various parts of the body. Later on Adrian,[4] and also Hügel and Holzhauser,[5] published successful experiments on transmission of syphilis to pigs, which developed exanthemata presenting the characters of specific papules. Neisser,[6] of Breslau, determined to check these statements by further experiments. He inoculated eighteen pigs under varying conditions, and in only a single instance did he obtain any skin lesion. In this case there was a circinate rash, with some resemblance to a secondary syphilide, but with marked differences on histological examination.

In consequence of the variable and unsatisfactory results obtained from experiments on the lower mammalia, the monkey was selected for observation on account of its biological affinity with man. Passing over experiments made at a time when hard and soft chancres were not differentiated, we shall epitomise, in a few lines, the most recent researches on monkeys. So far as we know, Klebs[7] was the first to inoculate the skin of a female guénon with portions of a hard chancre, which was followed by the development, six weeks later, of a papular eruption on various parts of the body.

In 1882 Martineau and Hamonic[8] inoculated a male macaque monkey, and four weeks later two hard chancres appeared on the prepuce, with the subsequent development of syphilides, glandular swellings and ulceration of the hard and soft palate. In spite of the positive results published by these writers their work was not followed up. This possibly arose from the absence of precise indication of the exact species experimented on. There is a large number of different species of macaque monkeys, which exhibit great diversity in their reaction to the syphilitic virus. Moreover, several observers have obtained negative or insignificant results from the inoculation of apes. Thus Sperk,[9] in 1886 and 1888, inoculated forty-six monkeys belonging to different species, and was only successful in a few instances. One subject, a male "macaque monkey," twenty-one days after the prepuce had been scarified with scrapings from syphilitic papule, showed an erosion which developed into an ulcer similar to a hard chancre. A month later there was a pustular eruption. With matter from the ulcer of this monkey Sperk obtained in another macaque a chancre-like ulceration, which took about three months to heal. In a third macaque inoculated from the second an ulcerated papule developed in fourteen days, which did not heal for fifty-two days after its appearance.

At about the same time Mossé, of Montpelier,[10] inoculated a young female cercopithecus with matter from a syphilitic chancre and from a mucous patch with a negative result. Many others, being equally unsuccessful, have not even thought it worth while to publish their investigations. For instance, it was only in 1903 that Krishaber, Fournier, and Barthelémy[11] announced that, as far back as 1882, they had inoculated macaques, "sajous," "ouistitis" and other monkeys. "The results furnished by these experiments," they say, "may be summed up in one word—nothing." We have been informed of equally unsuccessful experiments in the laboratories of Paris, of Breslau and St. Petersburg.

After this array of negative results we must mention the experiments made at the Pasteur Institute, in 1903, by Maurice Nicolle. While certain species proved insusceptible, one species of macaque did develop characteristic papules after inoculation over the eyebrow. These experiments, which M. Nicolle showed us at the time, have not been published. But more recently Dr. Charles Nicolle repeated them and proved that it is the bonnet monkey (*Macacus sinicus*) which is, to a certain extent, susceptible, three having been successfully inoculated. They presented squamous papules at the point of inoculation in from fifteen to nineteen days, which healed up in periods of from ten to twenty-three days. In one case only there was a subcutaneous induration with enlargement of glands. But in none of them were there any subsequent manifestations corresponding to secondary symptoms of syphilis. Still more recently Hamonic[12] communicated to the Academy of Medicine an experiment in which a Japanese macaque (*M. cynomolgus*) showed indurated ulcers with glandular enlargements, which commenced to heal nine days after their appearance.

From these data we may conclude that, as a rule, monkeys are immune to syphilis, but that in a few species certain syphilitic manifestations may be produced, which, however, are much less pronounced than in man.

We ourselves have inoculated several bonnet monkeys (*M. sinicus*) with syphilitic virus, and were able to confirm the results obtained by M. and C. Nicolle. About twenty days after inoculation papules developed locally with some œdema round them ; crusts formed on the papules which fell off in a short time. The neighbouring lymphatic glands were not enlarged to more than the size of a millet seed. The short duration of the primary manifestation and the absence of secondary phenomena indicated that the susceptibility of the bonnet monkey is very feeble, and a certain number (three out of five) showed complete insusceptibility. A young male mandrill (*Cynocephalus maimon*) was also insusceptible.

We then thought that the anthropoid apes, in view of their closer affinity to the human species, might be more susceptible. Comparative anatomy teaches that in every respect they are more closely allied to man than other monkeys are. This view, formulated particularly by Huxley, has been recently confirmed by Grünbaum and Nuttall in the course of their researches on the hæmolytic and agglutinative properties of blood serum. They found that the serum of animals reacts in exactly the same manner with both human blood and blood of anthropoid apes (chimpanzee, gorilla, orang-outang). Starting from these data we attempted to infect chimpanzees (*Troglodytes niger* and *T. calvus*) with human syphilitic virus. Our first experiment was made on a two-year-old female chimpanzee. We inoculated it on the prepuce of the clitoris with matter from a human hard chancre. At the end of a month the resulting lesion was already commencing to heal, and presented the cartilaginous induration of a chancre in the fold of the prepuce. (The patient from whom the matter was taken had enlargement of the sub-maxillary glands and a well-marked roseola. He had had no internal treatment, but had applied hydrogen peroxide solution locally.)

On the same occasion the chimpanzee was also inoculated over the right eyebrow with serum from a mucous patch taken from a patient who had a recent scar of a hard chancre on the penis, and who had three syphilitic ulcers on the same organ of three weeks' standing. Like the first patient he had had no general treatment, but had simply washed the parts with boracic lotion. As both these inoculations had been made from cases of some standing in which the lesions had been treated with antiseptics, we inoculated our chimpanzee five days later for the third time. This time we employed scrapings from a hard chancre of the penis only three days old which had not been treated. The inoculation was made on the left side of the preputial fold of the clitoris. In each case the inoculation punctures quickly healed without causing any permanent lesion. It was only on the 26th day that we perceived at the right side of the clitoris (the site of the first inoculation) a small, oval, transparent vesicle surrounded by a reddish, ill-defined margin. Soon the vesicle became flattened and broke, leaving an erosion surrounded by tissue which every day became more indurated. The floor of the chancre was at first ochre coloured,[1] but at the end of a short time the erosion was covered with a grey false membrane with a well-defined edge.[1] There was no enlargement whatever of the glands before inoculation, but at the time of the appearance of the local lesion glands could be felt in both groins and in a few days the enlargement on the right side (that of the inoculation) was very marked, a group of four glands being very distinctly felt. Neither these nor the smaller ones in the left groin were in the least tender to firm pressure.

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[1] Coloured plate in original.

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A month after the appearance of the chancre, *i.e.*, 56 days after the first inoculation, we noticed some papules on the white skin of the dorsal and ventral surfaces of the trunk as well as on the thighs. At first we could only discover four papules, but later on their number increased to fifteen. They were round, dry, squamous papules, slightly varying in size, with a red margin and a central crust. Slight scratching provoked the exudation of some pink and slightly turbid serum. As time went on these papules showed more and more resemblance to analogous lesions in syphilitic patients. Between the margin and the central crust developed a ring of small white scales, the *collerette de Biett*.[1]

* * * * *

Soon after the full development of these lesions the chancre began to heal; the red peripheral zone became pale and more and more infiltrated with black or brown pigment. The induration of the papules disappeared completely. In the mouth some ulcers appeared, but it was impossible to say that they were syphilitic, since chimpanzees in captivity are liable to obstinate ulceration of the gums. On the other hand, the generalised enlargement of the glands might more properly be regarded as a syphilitic manifestation. In addition to the glands in the groins already mentioned, those in the axillæ gradually enlarged. Moreover, during the development of the secondary manifestations the spleen enlarged and was hard on palpation.

About three months after inoculation the chimpanzee showed signs of illness. It was often found lying down in the cage, lost appetite and wasted. This condition persisted with remissions and relapses, but ultimately its health completely gave way. It remained lying all day, took scarcely any food and became rapidly weaker. Seventy-nine days after the eruption of the chancre, and forty-nine days after the first appearance of syphilides, it was found dead. The body only weighed 4,600 grammes. At the autopsy the inguinal glands were enlarged, especially those on the right side. The spleen, which weighed 40 grammes, was firm and hypertrophied, of a dark red colour, with many conspicuous Malpighian bodies. The liver was large (275 grammes), pale and yellow. There were several nodules on the surface. The kidneys were anæmic with a very well-developed cortex. The left lung was slightly œdematous and congested. In the mouth there were several sloughs round the teeth of the upper jaw. The epiglottis and glottis were very hyperæmic. Cultures from the heart blood, the liver, spleen and lung on various media showed abundant pneumococci, either isolated or in short chains. It is clear, therefore, that the chimpanzee died from a generalised pneumococcal infection, the organism having gained an entrance through the ulcers in the mouth.

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[1] Plates in original.

This put an untimely end to our experiment, which had, however, given some interesting results. It showed that the chimpanzee is much more susceptible to syphilitic virus than ordinary monkeys, and that syphilis developes in this animal much as it does in man. In addition to the slowly healing primary lesion there were secondary lesions in the form of papulo-squamous syphilides. From another point of view this first experiment proved that the human hard chancre, although a month old and in process of healing, contains enough active virus to produce syphilis in a chimpanzee. Thirdly, it follows from our experiment that immunity from the primary lesion is very quickly established, for of the three successive inoculations the first alone gave a positive result. The third inoculation, made five days after the second, was followed by no manifestation whatever, so that we may conclude that this immunity is acquired in very few days.

In order to determine whether syphilis in the chimpanzee can be transmitted to other individuals of the same genus, we inoculated a male chimpanzee with syphilitic matter from the first animal. Forty-five days after the appearance of the hard chancre in the latter, when the lesion was commencing to heal, we took some serum from it and inoculated the penis of the second animal superficially, using Vidal's scarifier. Reflecting that at this stage the virus might have lost its power, we simultaneously inoculated it on the left thigh with scrapings from a papular syphilide of the first animal. The small scarifications soon healed, except that at the end of eight days there were two trivial abrasions on the left thigh. It was only at the end of thirty-five days that a superficial erosion appeared on the left side of the penis, the size of a lentil, not indurated nor red. But in the following days the lesion increased in length and breadth. At the same time a second erosion with a slightly indurated edge developed on the thigh, and the glands could be felt in both groins. During the next few days the lesion on the penis made considerable progress. At the same time the primary lesion on the thigh ulcerated in the centre, became more red and indurated, and took on all the characteristic appearances of a superficial cutaneous chancre. The inguinal glands corresponding to the lesions on the penis and on the thigh underwent further enlargement, and two definite glands, moveable, indurated and hard, could be distinguished.

A month after its appearance the chancre on the thigh began to contract, while that on the penis underwent further development; and the left inguinal glands enlarged still more, but this increase was not maintained and their size soon diminished. The chancre on the thigh began to heal about six weeks after it had first shown itself, but that on the penis remained unchanged till the death of the animal forty-five days after the commencement of syphilitic manifestation. During its last few weeks the animal had a cold and coughed frequently. It steadily lost strength and appetite and ultimately succumbed. At the autopsy there was a typical hard chancre on the penis. The remains of the chancre on the thigh were surrounded by a pigmented margin, and the centre was covered with a hard, thick, dry crust. No secondary manifestations could be made out. The spleen was adherent to the parietal peritoneum; the liver and kidneys were pale and yellowish, and there was slight superficial ulceration in the stomach and ileum. Nothing abnormal was found in the lungs. Cultures on agar gave a very abundant growth of a small Gram-negative cocco-bacillus resembling the cocco-bacillus of Pfeiffer isolated from cases of influenza. But the two organisms are not identical, for that from the chimpanzee grows well on ordinary agar without the addition of blood.

The foregoing experiment furnished an additional proof of the susceptibility of the chimpanzee to syphilis. It demonstrated, moreover, that syphilis can be transmitted from one chimpanzee to another, and that a primary sore can be induced with equal readiness by inoculating virus from a hard chancre and from a papular syphilide. But whilst the virus of the chimpanzee produced syphilis in another individual of the same kind, it had no effect on a young mandril. Similarly scrapings from the chancre of the thigh of the second monkey inoculated over the eye-brow of a *Macacus sinicus* produced no effect. Possibly this may indicate an attenuation of the virus in passing through the chimpanzee. We are endeavouring to control this result by further experiments.

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Second Communication.

Annales de l'Institut Pasteur, Vol. xviii., No. 1
(January, 1904).

In our previous paper we demonstrated that the chimpanzee is susceptible to the virus of syphilis, which produces in this species of anthropoid ape primary and secondary symptoms exactly comparable to those in man. Quite recently Lassar, of Berlin,[1] has confirmed this, as he also has reproduced syphilis in a male chimpanzee. Syphilitic virus from an untreated hard chancre of the arm was inoculated in several places on the face and ears with the subsequent development of primary and secondary lesions. Fourteen days after infection there were several hard chancres at the sites of inoculation. At a later date characteristic papules made their appearance on several parts of the body, and notably on the palms and soles.

This experiment of Lassar and the two which we published show that out of three chimpanzees inoculated with syphilis all developed lesions comparable with those in man. Doubt, then, is no longer possible; the chimpanzee is an animal which can contract syphilis, and consequently may be of extreme value for the experimental study of the disease. The recent work of Neisser and Veiel[2] shows the importance attached by writers on syphilis to finding a susceptible animal. After unsuccessful attempts to remove the natural immunity of pigs temporarily by phloridzin and alcohol,[3] they attempted to convey syphilis to pigs and to a monkey which had been treated with "anti-cytasic" serum. The basis of their experiments is the fact observed by Wassermann that guinea-pigs treated with these forms of serum and inoculated with typhoid bacilli succumbed to generalised infection, while the control animals treated with normal serum resisted the same doses of poison. There is, however, this great difference between experimental inoculation with typhoid bacilli and with syphilitic virus, that in the latter case the incubation period is prolonged to several weeks, while typhoid septicæmia developes in less than twenty-four hours. It might have been foreseen, therefore, that the introduction of these "anticytasic" serums could in no way lower the resistance of the organism, and might even increase the natural immunity of an insusceptible animal. Moreover, in these experiments of Neisser and Veiel the pigs and the monkey (*M. pileatus*) did not show a single syphilitic symptom, though large quantities of the virus were introduced.

This negative result in a monkey has to be added to a large number of previously recorded unsuccessful attempts. In our own experiments on macaques with matter from human chancres by scarification (see p. 90) four only showed any results, and those quite trivial. Two (*M. sinicus*) presented slight lesions like those described by C. Nicolle.[4] In one of these the eyebrow which had been inoculated became the seat of a typical superficial lesion; two characteristic papules appeared on the upper lip, which had also been inoculated. These took the form of two red spots, the size of a pin's head, with a slightly scaly centre. The primary manifestations quickly cleared up and there were no secondary sequelæ.

Two (*Macacus cynomolgus*) have shown slight syphilitic manifestations. A female, inoculated on the upper lip and on the left eyebrow with virus from an early untreated chancre, showed a superficial lesion twenty-seven days later. A number of hyperæmic spots formed on the eyebrow and fused into a raised papule, on the centre of which a crust soon formed. This became pale and insignificant by the fourth day and soon disappeared. There was no manifestation on the upper lip, no enlargement of glands, and during the sixty-three days that the animal was kept under observation there were no secondary symptoms.

A second *M. cynomolgus*, inoculated on the left eyebrow and on the penis with virus from a human hard chancre, exhibited, twenty-eight days later, four discrete red spots over the left eye. Next day they were more marked and slightly scaly at the centre, but eight days later had almost entirely cleared up. The penis and glands showed no change. There were no secondary symptoms.

A third *M. cynomolgus*, as well as three *M. sinicus* and one *M. nemestrinus*, showed absolutely no reaction to syphilitic virus inoculated by scarification. Moreover, one *M. sinicus* inoculated subcutaneously in the thigh with matter from a hard chancre, and two *M. cynomolgus* similarly inoculated with blood from patients with syphilitic roseola, showed no signs of disease beyond slight glandular enlargement in one *M. cynomolgus*.

To recapitulate, out of twelve experiments made on macaques, four only developed lesions, and these trifling ones. The unimportant character of the syphilitic lesions, their short duration and the absence of secondary symptoms, have suggested that the poison becomes attenuated in macaques.

To clear up this point it was necessary to reintroduce the virus from the macaque into a susceptible animal, for which purpose we employed the chimpanzee. Immediately on the appearance of the primary lesion on the eyebrow of the first *M. sinicus* mentioned above, we removed some serous fluid and inoculated the prepuce of the clitoris in a young female chimpanzee which had been sent to us direct from the Congo. Vidal's scarifier was employed, and the slight erosions caused healed in a few days without leaving any traces. For a fortnight nothing occurred, but on the fifteenth day after inoculation the prepuce became hyperæmic, with a few spots redder than the parts round them. In the centre of one small red spot on the left side of the prepuce there was a tiny scale. On the right side we found another red spot, like a spot of roseola, with a central erosion the size of a pin's head. These lesions presented a striking resemblance with the round spots on the upper lip of the *M. sinicus* described above. By the next day the redness of the prepuce had greatly diminished, and the central crusts had become very dry and of a dark brown colour. The following day we found by the side of these fading spots three other red spots still smaller than the first. Five days after the first syphilitic manifestation the lesions on the left side of the prepuce had completely healed. The larger lesion was also in process of healing and was represented by a small dry crust surrounded by normal mucous membrane, and a day or two later this disappeared, so that the primary stage lasted only ten days. It is to be noted that the lesions excited in the chimpanzee by the virus from the macaque gave rise to no induration of tissue.

After allowing time for these lesions to heal completely we submitted our chimpanzee to a fresh experiment at the end of thirty days. It was inoculated on the prepuce of the clitoris with clear fluid from a hard chancre. The patient from whom it was taken had had a hard chancre on the penis for eight days and had painless enlargement of the inguinal glands on both sides, those on the right side corresponding to the sore being the larger. He had had neither local nor general treatment. As we did not wish to confine our experiment to the prepuce which had already been the site of lesions excited by virus from the macaque, we introduced some of the fluid from this patient into the left thigh, choosing this situation as it had been successful in the second chimpanzee described in our first communication (see p. 94). The inoculation was effected after superficial scarification of the skin. The trivial scarifications healed quickly without any reaction, but about eight days after the inoculation with human virus the chimpanzee exhibited general enlargement of glands. In both groins the glands which were not tender were easy to feel; in the axillæ the enlargement was less marked. In addition the posterior cervical gland could be made out as a small moveable body the size of a pea. During the ninety-three days which had elapsed since the inoculation with virus from the macaque no secondary lesions of the skin or mucous membranes had occurred, nor had any been exhibited during the sixty-three days after the introduction of human virus.

We may then fairly conclude that the first inoculation with virus from the macaque gave the chimpanzee immunity to syphilitic virus. It is impossible to attribute the absence of syphilitic lesions to natural immunity, for the first inoculation was followed by lesions, slight, but none the less characteristic. Nor is it possible to believe that the human virus inoculated as a check was inert, for the same virus was at the same time inoculated on a fresh *M. cynomolgus* which was to serve as a control, and also on a *M. sinicus* which had already had a primary lesion on the left eyebrow and on the upper lip. The "control" macaque showed, twenty-seven days after inoculation, a primary lesion on the eyebrow which we described above (p. 97). On the other hand the *M. sinicus* exhibited no symptom whatever, which proves that the first inoculation with syphilis, followed by a primary lesion, conferred immunity against fresh introduction of the virus.

These experiments, although few in number, suffice to demonstrate the possibility of obtaining an attenuation of the syphilitic virus by passing it through a macaque monkey, and producing an artificial immunity with the aid of the attenuated virus. But before we can dream of making a practical application of these ideas they will have to be confirmed more than once, and the whole subject more thoroughly investigated.

Seeing that the virus of the macaque, although attenuated, produced generalised glandular enlargement in our chimpanzee, a still milder virus must be sought for. Possibly the *M. cynomolgus*, which is more resistant than *M. sinicus*, will furnish it. Doubtless we shall be able to obtain from the ape family forms of virus with a descending scale of virulence, for the family includes species of different susceptibility, from the anthropoid whose susceptibility approaches that of the lower races of mankind, to the mandrills and maimons which show complete natural immunity.

In another direction search must be made for non-living vaccines obtained from syphilitic virus which has been acted on by various physical or chemical agents. Such investigations will require much time and many precautions. They are now in progress but are still far from giving definite results.

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Third Communication.

(*Annales de l'Institut Pasteur*, Vol. xviii., Nov., 1904.)

I.—SYPHILIS IN THE CHIMPANZEE.

AFTER having established in our first communication the fact that chimpanzees are susceptible to syphilis, and demonstrated in the second that the manifestations of syphilis in these anthropoids vary according to the origin of the matter inoculated, we have undertaken the investigation of the properties of the syphilitic virus and the influence on it of various factors. A portion of the results which are summarised in the present paper were communicated by one of us to the International Congress of Dermatology at Berlin in September, 1903.

There is no need to offer fresh proof in support of the groundwork of our investigations, viz., the experimental production of syphilis in anthropoid apes. Since we published our observations on the possibility of inoculating the chimpanzee with syphilis from man, and of maintaining its potency by passing it through these anthropoids, Lassar[1] succeeded in experimentally inoculating a chimpanzee with syphilis from a human hard chancre, and subsequently successfully inoculated a second chimpanzee[2] with syphilitic virus from the first. A little later Neisser[3] reported the results of syphilitic inoculations on several chimpanzees, some of which showed most typical primary and secondary lesions. In addition he inoculated several orang-outangs and a gibbon, which proved to be susceptible to syphilis, though to a less degree than chimpanzees.

Up to the present we have inoculated ten chimpanzees with syphilitic virus from different sources, and have obtained ten positive results, and Lassar succeeded in two instances. Twelve chimpanzees, therefore, have been inoculated and all of them have taken syphilis. That syphilis can be inoculated with certainty in certain monkeys is a most important fact in the experimental study of the disease.

Of the ten chimpanzees experimented on by us, seven were inoculated with human virus of different kinds. All of these were inoculated with serum from hard chancres occurring in several individuals, and four were inoculated in addition with matter from secondary lesions (mucous patch and a chancriform syphilide). One chimpanzee was inoculated with matter from a chancre and from a papular syphilide of another anthropoid; another was treated with serum from the primary sore of a *M. sinicus*, while another was inoculated with matter from a chancre of a *M. cynomolgus*. Chimpanzees are susceptible, as we have already said, to virus from all these sources.

The inoculation was effected in almost all cases with a Vidal's scarifier, by numerous very superficial scarifications, which were made either over the eyebrow, on the eyelids, or on some part of the genital organs—the clitoris or its prepuce, or on the penis or its prepuce. In a few experiments we injected, in addition, some of the virus subcutaneously into the thighs.

The length of the incubation period, when the virus was derived from a human subject, varied between twenty-two and thirty-seven days (in our seven cases the dates were twenty-two, twenty-two, twenty-six, thirty-three, thirty-five and thirty-seven days). The primary lesion commenced as a small spot, scarcely deeper in colour than the parts around, and slightly raised.[1] At the onset these features were sometimes so indefinite as to warrant doubt as to their significance, and it was only at a later stage that their syphilitic character was beyond dispute. There was never a sharp line of demarcation surrounding the round or oval spots. On two occasions only we observed vesicles containing fluid on these spots. On our first animal there was a clear vesicle on the prepuce of the clitoris, and in a later experiment two initial spots on the eyelid of a chimpanzee were surmounted the next day by grey, opaque vesicles.[1] Soon after their appearance the vesicles became flattened and altered into crusts, at first small, but later steadily increasing in size. With the exception of these two anomalous cases vesicles never developed. On the day after their appearance the small rose spots showed tiny scales in the centre. These scales were later on transformed into yellow or brown crusts, which became larger and cracked, allowing a little clear serous fluid to ooze out. At the end of a variable number of days these lesions developed into very characteristic hard chancres.[1] The edge was raised, and the floor of the sore, after the scab fell off, was moist, pale, and of a translucent red colour (lardacée). The chancres were usually multiple, and developed in some cases at different sites of inoculation, such as the supra-orbital arch, the eyelid and the thigh. They lasted for months, the healing process taking place very slowly over a long period, which varied in length in different experiments. A few days after the appearance of the primary lesion the neighbouring lymphatic glands enlarged. They were freely movable and not tender.

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[1] These are the figures in the original. One has apparently been omitted.—*Trans.*

It is clear, from this description, that the primary lesion in the chimpanzee corresponds in all respects with that in man. This primary lesion is followed by secondary manifestations, also comparable with those met with in the human subject. We have already described (p. 92) the papulo-squamous syphilides which developed on various parts of the body in our first chimpanzee. Lassar observed similar papules on the head, the arms, and particularly on the soles of his two chimpanzees. They developed a month after the appearance of the chancre, and presented all the characters typical of analogous lesions in man. The syphilitic nature of this cutaneous affection was quite evident. but, to remove all doubt, we inoculated a second chimpanzee with scrapings from a papular syphilide of the first, and, as already related (p. 94), with a positive result.

Since that time we have observed secondary symptoms in two other chimpanzees. In one of them two small superficial erosions, with sharply defined margins, appeared on the tongue eighteen days after the commencement of the chancre. They were followed in two days by two fresh erosions of a deeper red colour than the rest of the tongue. They were very slow in healing, and could be distinguished for more than six weeks. Forty days after the commencement of these secondary symptoms two characteristic red mucous patches, with a pale border, appeared at the tip of the tongue, and soon after these a perfectly typical mucous patch on the lower lip.[1] In another chimpanzee four syphilitic papules appeared on the face twenty-nine days after the commencement of the primary lesion and sixty-six days after the inoculation. They healed in about a fortnight, leaving well-marked pale cicatrices. The syphilitic nature of these lesions was proved by inoculating two macaques (*M. sinicus* and *M. cynomolgus*), with scrapings from one of the first erosions on the tongue, when characteristic primary lesions followed. Histological investigation of the lesions in chimpanzees were made, in the first instance, by Becker and Meyer,[4] with the material furnished by Lassar, and subsequently by Arnal and Salmon, with specimens from our animals. These observers demonstrated a close resemblance with the lesions of human syphilis. Except where they were modified by secondary infections, the lesions were characterised by a large aggregation of mononuclear cells and typical periarteritis. In a few of our syphilitic chimpanzees the spleen was enlarged during the secondary stage, and the enlargement was maintained for a considerable time, but we observed no other lesions of the internal organs. In the chimpanzee which showed mucous patches of the tongue and lower lip, paraplegia developed and lasted for more than a month. Possibly this must be attributed to a generalised syphilitic infection.

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[1] Coloured plate in original.

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[1] Coloured plate in original.

II.—THE PROPERTIES OF SYPHILITIC VIRUS WHEN FILTERED.

Having established these general facts, it was important to study the properties of the virus itself. Our microscopic investigations failed to give any satisfactory result. Minute examination of the serum from the initial vesicles revealed the presence of leucocytes and of some red corpuscles, but we were unable to distinguish any organisms. The fine granules in the liquid (evidently *débris* from broken-down cells) did not betray any movements which could be attributed to mobile organisms in their neighbourhood, such as spirilla, nor, in these specimens, could spirilla be demonstrated by staining with neutral red, which stains the spirilla of birds so readily. It might, then, have been supposed that we were dealing with one of those invisible organisms which we are led to assume in the case of certain infectious diseases, such as yellow fever. But the recent experiments of Klingmüller and Baermann[5] seem to negative this view. They inoculated themselves with syphilitic matter from man which had been triturated with normal saline and filtered through a Berkefeld filter. The result of several successive inoculations was absolutely negative, from which they concluded that the virus was retained by the filter. But the objection may be raised that there was no control experiment to prove that the syphilitic matter treated in the same way, but not filtered, was actually capable of communicating syphilis. The delay of several hours necessary for obtaining the filtrate, or even the normal saline employed for the dilution, might possibly be capable of modifying the virulence. Since it is inadmissable to make use of a human being for such a control experiment, the experiments can be much better made on the anthropoids, for though less heroic, they are more conclusive and more precise than those which we are able to make on man.

We, therefore, took virus from hard chancres on the penis of two men and from two syphilides in a woman, and diluted it with 2 cc. of aqueous humour taken from a sheep immediately after it was slaughtered. The mixture was filtered through a Berkefeld filter (12A), and a little of the filtered liquid was inoculated on the orbital arch and on the thigh of a fresh chimpanzee with a Vidal's scarifier. Only one or two drops were used for these superficial inoculations ; the remainder, that is almost all, of the filtrate was injected subcutaneously into the thigh of the same animal The whole operation from the time of taking the syphilitic matter from the subjects to the inoculation did not take more than fifty minutes. The filter employed had been thoroughly tested by M. Dujardin-Beaumetz, who found that it allowed the organism of bovine pneumonia to pass but prevented the passage of bacteria, such as cholera vibrios.

The inoculation of this filtered virus was followed by no pathological manifestation ; there was no lesion, syphilitic or other. To satisfy ourselves that this negative result could not be attributed to alteration of the virus by the aqueous humour of the sheep, or by the time necessary for the filtration of the liquid, we made a control experiment on another chimpanzee, which received the same virus diluted with the same aqueous humour in the same sites as the first, the only difference being that the mixture was not filtered. In this case, on the thirty-seventh day after inoculation, three round prominent spots appeared over the eyebrow, which quickly developed into three typical hard chancres. Soon afterwards the gland beneath the angle of the jaw on the same side was found to be enlarged. A few days later two typical hard chancres developed on the thigh. The deduction from this experiment, performed under strict conditions, is therefore in agreement with that of Klingmüller and Baermann—that syphilitic virus cannot pass through the Berkefeld filter, although that filter permits the passage of the virus of bovine pneumonia.

III.—PROPERTIES OF SYPHILITIC VIRUS AFTER TREATMENT BY HEAT AND GLYCERINE.

Filtration is obviously not the only means by which the virulence of syphilitic virus might be abolished. Since similar poisons are generally sensitive to the action of more or less elevated temperature, we naturally tried to determine to what temperature the virus of syphilis had to be raised in order to deprive it of all pathogenic action. We shall only relate one of the experiments. We employed human syphilitic virus mixed with aqueous humour from a sheep. We introduced 2 cc. of the mixture which served to inoculate the "control" chimpanzee in our filtration experiment into a sealed tube, which was incubated for an hour at 51° C. Immediately afterwards a few drops of this liquid were inoculated by scarification over the eyebrow, on the eyelid, and on the thigh of a fresh chimpanzee, whilst the rest, *i.e.*, the bulk, of the liquid was subcutaneously injected into the thigh of the same animal. The result was absolutely negative, proving that exposure for an hour to a temperature of 51° C. suffices to destroy the virulence of syphilitic virus.

The low resistance of the virus to heat would make one expect that it would be equally sensitive to the action of chemical agents, and to settle this question we made use of syphilitic virus mixed with glycerine. A few drops of matter taken from a hard chancre on the penis of a man who had eleven chancres, were mixed *in vitro* with several volumes of concentrated glycerine. This mixture was immediately inoculated with a scarifier on the upper eyelid, the orbital arch and the vulva of a young female chimpanzee. The scarifications quickly healed, but thirty-three days after the inoculation three slight lesions appeared over the orbital arch and in the course of a few days developed into perfectly hard chancres. In another similar experiment virus from a human hard chancre, mixed with glycerine, gave rise to a typical secondary lesion, thirty-five days after inoculation, in a chimpanzee. Glycerine, therefore, added to a syphilitic virus under the conditions we have described, does not remove its pathogenic potency.

Seeing that infective material, although deprived of its virulence, has often the power of protecting a subject against the corresponding disease, it was natural to inquire if the syphilitic virus, after filtration or heating at 51° C., might not have been transformed into a vaccine. With this in view we submitted the two chimpanzees mentioned above to a test inoculation. The one which had been treated with filtered virus was inoculated three weeks later over the eyebrow and on the upper eyelid with a little virus from a hard chancre on the penis of a man which had appeared five days before, the glands not being yet enlarged. Immediately afterwards the same monkey was inoculated on the thigh by means of a scarifier with matter from a hard chancre of another man. Twenty-two days afterwards we noticed a small rose spot above the eye. At the same time the place on the thigh which had been scarified became reddened, without, at first, any swelling or induration. But in a few days there could be no doubt of the syphilitic nature of the lesions. The rose spot above the eye joined with another similar spot to form a hard chancre covered with a thick crust. At the same time six small chancres developed in the red patch on the thigh and quickly fused into a single large hard chancre. The corresponding glands near the jaw and in the groin showed considerable enlargement.

The second chimpanzee, which had been treated with virus which had been kept at a temperature of 51° C., was inoculated, after an interval of twenty-one days, over the orbital arch, on the upper eyelid, on the thigh, and on the penis, with human syphilitic virus from the same cases as in the experiment just described. Thirty-three days after this test inoculation two faint rose spots made their appearance on the upper eyelid and developed into two typical hard chancres, whose appearance was soon followed by enlargement of the corresponding glands.

These two experiments compel the conclusion that syphilitic virus, either filtered or kept at a temperature of 51° C., under the conditions we have described, are incapable of protecting against the primary lesion. Perhaps, generally speaking, infective material, which has been deprived of all pathogenic activity and is incapable of provoking the slightest local lesion, is unsuitable for conferring immunity against syphilis. A few facts which we have observed support this supposition. A chimpanzee was noculated with virus obtained from a *Macacus cynomolgus*. The quantity of virus taken was very small and was mixed with blood. The result was absolutely negative, so that forty-eight days later a second inoculation was performed with syphilitic matter from another *M. cynomolgus*. This time a larger quantity of virus, inoculated in sites not employed in the previous experiment, gave rise, forty-nine days later, to two chancres, one over the superciliary ridge and the other on the upper eyelid. Their syphilitic nature was confirmed by the subsequent development of great enlargement of two glands behind the angle of the jaw on the corresponding side. These glands were movable and not tender, and fluid from them produced definite primary lesions in three macaque monkeys (*M. cynomolgus*). The chimpanzee under observation, therefore, had not been protected by the first inoculation with virus from a macaque, the inoculation having led to no local reaction. This fact indicates once more that an anti-syphilitic vaccine must be sought amongst forms of virus which are capable of exciting local lesions, which should, of course, be as slight as possible. It is evident that syphilitic virus from this species (*M. cynomolgus*) is incapable of serving for this purpose. If inoculated in a small dose it gave rise to no phenomena at the site of inoculation, while in too large a dose it provoked far too severe symptoms. The chimpanzee, whose case we have partially related, showed at a later date serious secondary symptoms, viz., mucous patches on the tongue and lower lip.

From our experiments it would appear that the virus is attenuated to a much greater extent by passing through another kind of monkey, the Chinese bonnet monkey (*M. sinicus*). In our second communication (p. 98) we related the history of a chimpanzee which was inoculated with virus from this species.

IV.—SYPHILIS IN THE LOWER CATARRHINE APES.

In the search for a method of vaccination against syphilis in which attenuated living virus may play a considerable part, it is important to obtain information on the manifestations of syphilis in the lower apes. Up to the present we have employed apes of the Eastern hemisphere, the catarrhines, on the supposition that the platyrrhines of the New World being more remotely related to man might enjoy greater immunity from syphilis.

A short-tailed species of macaque (*M. rhesus*) is susceptible to some extent to syphilis. One only, out of three inoculated with human virus on various parts of the body, exhibited a hard chancre above the eyebrow 23 days after inoculation. This primary lesion healed up within three weeks and was followed neither by enlargement of glands nor by any secondary symptoms whatever.

Long-tailed macaques are more susceptible. The occurrence of the primary lesion in bonnet monkeys has been demonstrated by Maurice and Charles Nicolle. Out of twenty monkeys of this species observed by us, ten only (50 per cent.), have exhibited lesions at the point of inoculation, viz., a chancre slightly indurated or not indurated at all which showed a tendency to heal rapidly. Slight enlargement of glands was observed in a few cases only. The old monkeys were usually resistant, and one very young monkey (only a few months old) showed complete immunity.

One *M. cynomolgus* has been inoculated successfully by Hamonic. In our own experiments it has been much more susceptible than the bonnet monkey. Ten monkeys of this species out of fifteen inoculated by us (*i.e.*, 66 per cent.), showed primary lesions analogous with those in the bonnet monkey. We succeeded in producing chancres at the point of inoculation and occasionally enlargement of glands, but never secondary lesions. On one occasion a pigmented cutaneous affection, resembling a patch of lupus, developed in the neighbourhood of the chancre. As to the nature of this cutaneous lesion there was conflict of opinion, some authorities pronouncing it syphilitic, while others, including Professor Fournier, regarded it as a secondary affection of the skin, but not syphilitic.

A "magot" (*Inuus ecaudatus*), a *Cercopithecus pathas* and a *Cercopithecus callitrichus* inoculated by us with human virus showed complete immunity.

Among the cynocephalous apes one young mandrill (*C. maimon*) was unaffected by virus from a syphilitic chimpanzee, although a young hamadryad (*C. hamadryas*) inoculated with serum taken from syphilides of a woman presented typical lesions. After an incubation period of thirty-five days small red spots covered with dry scales formed at the site of inoculation over the eyebrow. This lesion increased for some time and took three weeks to heal, leaving a dark pigmented trace behind. For some time a small gland could be felt below the angle of the jaw on the same side, but there were no secondary symptoms during the four months that the animal was under observation.

In a recent paper Zabolotny[6] has described syphilitic lesions obtained in a baboon (*C. sphinx*) with human virus, viz., a hard chancre at the site of inoculation on the penis and a secondary roseolar and papular rash. He inoculated four monkeys of the same species successively from one to another, and demonstrated in them the same primary and secondary manifestations. We ourselves began by inoculating two baboons (*C. sphinx*) over the orbital arch, on the eyelids and on the genitals with human virus. In one of them, two weeks after inoculation, there was a reddened patch on the upper eyelids covered with slight superficial scales. There was neither induration, swelling of the eyelids, nor any enlargement of glands. The primary lesion cleared up in three weeks leaving a dark pigmented trace. At the end of three months no secondary manifestations had appeared. The second inoculated with virus from mucous patches on the penis and lip of a man was under observation for eighty-nine days, but showed no symptoms whatever. Two other baboons of the same species, kindly placed at our disposal by M. Laveran[1] showed more definite syphilitic lesions.

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[1] These two animals had proved in M. Laveran's experiments to be immune to trypanosomes of all kinds. It is then hardly likely that the organism of syphilis is a trypanosome, as might have been imagined from the clinical analogy between syphilis and the dourine of horses.

Seventeen days after inoculation with human virus two of them exhibited hyperæmic patches on the upper eyelids, which were covered with numerous scales.[1] There was no induration, œdema, nor glandular enlargement. Healing took place in less than three weeks and no secondary symptoms were observed during three months.

So far as our observations go, therefore, syphilitic lesions in the cynocephalous apes more closely resemble those in macaques than those met with in the anthropoids and in man.

The study of all these varieties of the disease is of great importance in connection with the attempt to combat syphilis in the human race. If it is legitimate to draw any inference from our observations, it would seem that attenuation of the syphilitic virus for the purpose of procuring a vaccine is most likely to be effected by passing the virus though the lower catarrhine apes. If the virus obtained from bonnet monkeys is too strongly pathogenic we shall have to have recourse to less sensitive species, such as *M. rhesus.* In order to regulate the toxic effect it may be well to combine the use of attenuated virus with the employment of a specific serum.

Notwithstanding the doubts which have been expressed by Neisser we are absolutely persuaded of the syphilitic nature of the experimental lesions in macaques. Their transmission to chimpanzees and the primary and secondary manifestations in the latter, compel us to adopt this view.

Ever since the researches of Richet and Hericourt repeated attempts have been made to prepare antisyphilitic serum, but up to the present with only negative results. It is possible that the experimental study of syphilis in monkeys may throw light on this question. Species which show some reaction to syphilis, although feeble, may furnish more active serum than that supplied up to now by species which are unaffected by the virus. It must not be forgotten that the study of syphilis in animals has only just commenced, and that following on the preliminary researches which we have just related there remains a vast field for experimental enquiry.

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[1] Plate in original.

REFERENCES.

[1] Lassar. *Berlin klin. Wochenschr.*, 1903, p. 1,189.

[2] Lassar. *Ibid.*, 1904, p. 801.

[3] Neisser. *Deutsch. med. Wochenschr.*, 1904, pp. 1,369 and 1,431.

[4] Becker and Meyer. *Berlin klin. Wochensch.*, 1903, p. 1,192.

[5] Klingmüller and Baermann. *Deutsch. med. Wochenschr.*, 1904, p. 766.

[6] Zabolotny. *Arch. des Sciences Biologiques*, vol. xi., p. 155 (Russian edition).

Fourth Communication.

(*Annales de l'Institut Pasteur*, Vol. xix., Nov., 1905.)

A YEAR ago we contributed to this Journal a *résumé* of our investigations on syphilis. Since that time we have continued our researches, and although our work is far from complete, we present the results for the benefit of the numerous workers in this field.

Experiments on anthropoid apes are particularly difficult on account of the delicacy of these animals and of the length of time the observations demand. Experiments on the lower apes are more convenient, but the results are not so satisfactory owing to the absence of secondary symptoms and to the discrepancies between manifestations of syphilis in these animals and in man. Under these circumstances, publication of our results may be useful to those who are working at the same subject, and will save them from undertaking lines of investigation which have already been worked at.

We hope also to demonstrate by this paper that we have made the best possible use of the grants which have been placed at our disposal by generous friends. Experiments on anthropoids are very costly, and the resources of the Pasteur Institute would not have enabled us to undertake them. They have been prosecuted, thanks to the important sum furnished by the Osiris Prize, supplemented by a grant of £1,200 given by Mme. Varwara Morosoff and M. Jean Morosoff of Moscow. The Society of Dermatology and Syphiligraphy of Moscow also made us a grant of 250 roubles. To all these generous donors we desire to express our sincere gratitude.

I.—SYPHILIS IN ANTHROPOID APES.

Wider investigations on various representatives of the simian group have established the fundamental fact that it is in the chimpanzee that the manifestations of syphilis most closely approximate to those in man. Susceptibility in this animal is complete. Out of twenty-two inoculations of separate chimpanzees with syphilitic virus from different sources, we have not had a single failure; all have shown typical manifestations of syphilis. The incubation period has varied from fifteen to forty-nine days, the average being thirty days.

The first appearance and the subsequent evolution of the primary lesion have already been sufficiently described. We may mention that in every case the primary lesion has been followed, after a few days, by enlargement of the neighbouring lymphatic glands. In some cases the enlargement extended to distant glands. At the autopsy (made by Dr. Weinberg) on one chimpanzee, which had exhibited syphilitic papules and palmar psoriasis, numerous enlarged glands were found along the abdominal aorta and the iliac arteries. In our earlier papers we showed that chimpanzees are subject to secondary syphilitic lesions, such as papules and mucous patches. We do not venture to be positive as to the significance of roseola in syphilitic chimpanzees, as an exactly similar eruption was observed in several chimpanzees which had not been inoculated.

Of our twenty-two chimpanzees, eight presented secondary symptoms, three had mucous patches on the lips, and seven (two of whom had mucous patches) showed papules, either dry or with a broken surface. Several were affected with characteristic palmar psoriasis.

The interval between the appearance of the primary lesion and that of papules or mucous patches, varied from nineteen to sixty-one days, with an average of thirty-three days and a half. In our eight cases the numbers were: 19, 21, 24, 29, 30, 60 and 61.

Of the fourteen chimpanzees which did not show secondary lesions, four were inoculated from Chinese bonnet monkeys (*Macacus sinicus*) whose virus shows a certain amount of attenuation. One of these lived sufficiently long (one hundred and eighteen days) for secondary lesions to have shown themselves. Of the other three, one died twenty-seven days, and a second eleven days, after the appearance of the chancre, while the third is still alive, forty-two days after the first manifestation of syphilis. Four others which died without secondary symptoms appearing lived less than the average time (thirty-three and a half days) which elapses between the appearance of primary lesions and those of secondary symptoms, dying respectively nine, twenty, twenty-nine and thirty days after the appearance of the chancre.

Consequently only four chimpanzees remain which lived for a sufficient time (thirty-eight, forty-five, forty-eight and eighty-one days), and yet presented no secondary symptoms. One of these was inoculated with virus from a macaque (*M. cynomolgus*), and had been previously inoculated from a chimpanzee with virus, whose properties had been destroyed by heat (see p. 26). Another, having lived forty-five days, was inoculated with fresh virus from a chimpanzee. The third, inoculated with human virus, was treated with virus from a baboon which had been immunised against syphilis. The fourth, having shown no secondary symptoms, was at first treated with human virus which had been filtered through a Berkefeld filter (see p. 107), and was later on inoculated with virus from a human hard chancre.

So that, even if anyone declines to attribute the absence of secondary symptoms in these animals (which had lived for a sufficient time) to the different procedures detailed above, there remain only four animals to set against the eight which did present secondary symptoms of various kinds. Chimpanzees would appear, therefore, to be subject to these accidents in the proportion of sixty per cent. In most of our cases the secondary lesions were neither extensive nor severe: scanty or ulcerated papules disseminated over the whole body, or a few mucous patches in the mouth. Each kind of lesion persisted for some weeks, and then healed up leaving cicatrices.

In only a few instances were the secondary lesions severe. In one chimpanzee, inoculated over the eyebrow, on the eyelids and on the thigh with human virus, a series of chancres developed on the left thigh on the thirty-seventh day. Twenty-nine days later four papules appeared on the face and healed up in a fortnight, leaving well-marked, non-pigmented scars. Soon afterwards more prominent brownish papules appeared on the skin of the abdomen; some in groups, others isolated. In the course of a few days they became more numerous. They were covered with thick scales. There was no serous exudation and on scratching them deeply only a little blood-stained fluid was obtained. During the following weeks secondary symptoms became aggravated, and the hair fell off all over the body. The denuded skin showed numerous papules covered with white scales, which afterwards ulcerated and could be seen from a distance. For some time the general health was unaffected, then progressive loss of weight and strength set in. Professor Fournier, who was kind enough to see the animal, stated it was the first case of severe syphilis he had seen in an animal and he thought it would prove fatal in a few days. This prognosis was justified, for the animal died six days after in a state of profound cachexia.[1] Bacteriological examination of the ulcerated papules showed a number of different bacteria with a predominance of cocci. It is very probable that death was due to secondary infection by these organisms. This view is supported by the fact that a second chimpanzee which lived in the same cage had similar cutaneous lesions at the same time.

This second animal had been inoculated from primary lesions of a baboon (*Cynocephalus sphinx*) and treated with blood-serum of monkeys immunised against syphilis. These injections did not prevent the appearance of a typical primary lesion on the forty-fifth day after inoculation. The hard chancre developed in the ordinary way, and twenty-four days later was followed by a papular eruption on the skin of the abdomen. During two months the spots increased in number and, though less abundant than those in the animal just described, they covered the greater part of the body. With a prominent rim and a flat top covered with a crust these papules resembled small chancres. They then ceased to increase in size, and a month later had completely healed. Serum from two of these papules, obtained by scarifying, was inoculated over the eyebrow of a baboon (*C. sphinx*). After nineteen days typical primary lesions resulted, which developed in a normal manner and healed up in ten to fifteen days. This result confirms the syphilitic nature of the papules of the chimpanzee.

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[1] Photographs in original.

In addition to secondary lesions in the skin and mucous membranes we have several times observed nervous symptoms in chimpanzees. For example, in the animal described above which died of malignant syphilis, paresis of the hind feet lasting three weeks developed simultaneously with the first papules on the face. As in similar cases this nervous disturbance passed of completely. Another chimpanzee inoculated with virus from a macaque (*M. cynomolgus*) also had difficulty in walking during the period of development of mucous patches in the tongue and lower lip. Although its general health did not suffer, and its appetite and spirits were good, it had difficulty in dragging its feet along. We asked Professor Nissl of Heidelberg, who was visiting us, to examine the chimpanzee. He found that there was paraplegia. The right knee-jerk was active, but that on the left side was very feeble. During the following weeks matters improved. For some time it could only drag its feet, but, later on, weakness was confined to the right foot. Finally, seven weeks after the onset of paraplegia, the gait was normal.

There was enlargement of the spleen in several of our chimpanzees. Almost all of these died of broncho-pneumonia, and the autopsies showed no lesion of internal organs which could with certainty be attributed to syphilis. Nothing was found in any of our animals that could be regarded as a tertiary symptom.

From the facts we have summarised it appears that the chimpanzee is the animal which best lends itself to the experimental study of primary and secondary syphilitic lesions. Three gorillas which we were able to procure lived for too short a time to give positive results, and our experience with three orang-outangs showed that this animal is less suitable than the chimpanzee.

One of our orang-outangs inoculated over the eyebrow and on the eyelids with human virus, manifested twenty-three days later characteristic primary lesions with bilateral enlargement of the sub-maxillary glands. The chancres healed up in two months, but no secondary lesions developed during the remaining sixty-nine days of the animal's life.

A second orang-outang, inoculated on one side with virus from a macaque (*M. sinicus*) and on the other with human virus, presented primary lesions over each eyebrow at the end of twenty-six days. They began as herpetiform vesicles and in the course of a few days gradually developed into typical hard chancres. Enlargement of the retro-maxillary glands occurred a fortnight after the eruption of the local lesion. Although this animal lived for eighty-nine days after the appearance of the chancre no secondary lesions were to be seen either in the skin or mucous membranes.

A third orang-outang (female) was inoculated over the eyebrows and on both eyelids with virus from a human hard chancre. Immediately after inoculation the animal was treated with serum from baboons immunised with blood from a syphilitic patient. Nevertheless, twenty-two days later a small primary lesion made its appearance under the form of a herpetiform vesicle, which afterwards became flattened and transformed into a crust, thin at first, but afterwards thick. The chancres on each side were of insignificant size and healed in six weeks. There was enlargement of retro-maxillary glands on both sides. The only secondary manifestation was slight palmar psoriasis of both hands, appearing three and a half months after the primary lesions and clearing up in a short time. No other lesions appeared during the nine months that the animal lived after the chancre was first seen. Their absence cannot be attributed to the injections of serum, which did not prevent the primary lesion, and which were stopped immediately after its occurrence.

The orang-outang, therefore, in its relation to syphilis has shown itself further removed from man than the chimpanzee, and, consequently, we afterwards confined our observations to chimpanzees.

Seeing that, even in this group of apes most closely allied to man, there are classes which are but little liable to the generalised effects of syphilis, it is not surprising to find complete absence of secondary symptoms in the lower catarrhine apes. Out of 120 macaques and baboons inoculated with syphilitic virus from different sources, we have never observed true secondary lesions. In a very small number of cases we have observed a cutaneous lesion similar to lupus in the neighbourhood of the primary sore after this had healed. Inoculation in guinea pigs was negative, so that the view that this was a secondary tuberculous infection was not confirmed. We described a similar case in our third communication (p. 111), and since then Finger and Landsteiner,[1] of Vienna, have observed two similar cases. Even enlargement of glands in the neighbourhood of the chancre is rare in the lower catarrhine apes. In the great majority of cases there is no enlargement at all, in a few there is slight swelling, which quickly disappears.

Under these conditions it is not surprising that the possibility of producing syphilis in these animals has so long escaped recognition. The statements of some authors as to secondary lesions in macaques and baboons were not confirmed, and the primary lesion was often too insignificant to attract attention.

At the present time it is fully established that the lower catarrhine apes do actually present primary syphilitic lesions, although these are not severe. Neisser,[2] who for a long time was unable to accept this conclusion, has ultimately adopted it.

II.—Attempts at Preventive Treatment.

Having demonstrated that monkeys could contract syphilis it was natural to search for preventive measures. Prolonged experimental investigation has convinced us that this will be far from easy.

Our first experiments having shown that a fresh injection of virus five days after a previous one was without effect, we did not hesitate to make test inoculations at a short interval after inoculations supposed to be preventive.

But in course of time we were led to alter our opinion, being led to do so by the following facts :—

One of our orang-outangs was inoculated at the outset with virus from a macaque (*M. sinicus*). Ten days later it was inoculated in another part of the body with human virus. We fancied that the virus of the macaque might have a vaccine-like influence and prevent the formation of a chancre at the point where the human virus was introduced. But what actually occurred was that twenty-six days after the first inoculation a chancre developed over the eyebrow, the site of inoculation with simian virus. But this did not hinder the production of a second chancre (after an incubation of twenty days) from human virus over the other eyebrow. Immunity, therefore, was not established in the ten days which elapsed between the two inoculations. In another experiment, human virus inoculated eighteen days after virus from a macaque, also gave rise to a primary local lesion.

M. Queyrat[3] has shown clinically in man that even after the appearance of a chancre, that is, three to four weeks after infection, auto-inoculation of syphilis sometimes succeeds. He convinced himself that "immunisation against syphilis is not complete ten days after the appearance of the chancre" (p. 9). In view of these observations we decided to delay test inoculations till six or eight weeks after the first introduction of the virus.

Immediately after the first applications of serum therapeutics, attempts were made in different places to prepare some form of anti-syphilitic serum. Syphilitic matter was injected into different animals in the hope that their serum might become capable of curing syphilis. Others employed, both as a curative and preventive agent, blood serum of patients who had been attacked by syphilis some time before. The results gave so little encouragement that serum treatment of syphilis has been abandoned almost everywhere. So far as we know the method is now employed only in the wards of Prof. Maragliano, of Genoa.[4] We thought that possibly the lower apes, which adapt themselves to our climate, easily contract the primary lesion and quickly recover, might furnish a more active serum than that obtained from horses and other domestic animals. We commenced by cutaneous inoculations of macaques (*M. sinicus* and *M. cynomolgus*) baboons (*C. hamadryas* and *C. sphinx*). When the chancres had healed we gave repeated subcutaneous injections of blood taken from syphilitic patients with an abundant roseolar rash. It is well established by observations on man, that in these conditions the blood contains the virus, and we have confirmed this by injecting macaques with blood from a chimpanzee at the time when secondary symptoms were fully developed. But although some of our animals were immunised more than fifteen months ago the results hitherto obtained have been inconclusive.

In the previous chapter we mentioned incidentally some examples of syphilitic symptoms occurring in anthropoids (chimpanzee and orang-outang), although they had been treated with serum. In all our experiments serum injected under the skin has proved inefficacious. The following is one of several examples :—

A chimpanzee which had been inoculated over the eyebrow with virus from the chancres of two baboons (*C. sphinx*), was treated immediately afterwards by injections of blood serum from one macaque (*M. sinicus*) and four baboons which had all been immunised. The macaque, after having had a chancre due to inoculation with human virus, had received, during the course of four months, 62 cc. of blood from patients with an abundant syphilitic roseola. The four baboons had been inoculated with virus from human chancres ; three had typical primary lesions, in the fourth no lesion followed. When the chancres had healed repeated subcutaneous injections of blood from patients with syphilitic roseola were made over a period of seven months. The unsusceptible baboon received 105 cc. and the three others 108·5 cc., 88·5 cc., and 73·5 cc. respectively. The injected blood was absorbed in a very few days. Blood was drawn from the macaque and the baboons on repeated occasions, and their serum, which had been heated for an hour at a temperature of 57° C., was employed for the treatment of the chimpanzee. But although treatment was commenced on the same day as the inoculation, and as much as 55·5 cc. was injected in the course of thirty-eight days, the chimpanzee developed a primary sore on the forty-fifth day after inoculation. The chancre was very large and did not yield to repeated injections of serum. A mass of enlarged glands also formed behind the ear in spite of the treatment. Twenty-four days after the appearance of the chancre, when the chimpanzee had received, in all, 76·5 cc. of serum from the macaque and the baboons, syphilitic papules came out freely on the abdominal wall. In other words, in this animal serum treatment proved incapable of preventing the primary and secondary symptoms of syphilis.

We then tried to discover whether this serum, which had no effect on virus already introduced into the system, would have any action on the virus *in vitro*. We mixed virus from chancres of two patients with serum from a baboon which had been receiving syphilitic blood subcutaneously for eight months. We inoculated a chimpanzee with this mixture. It lived for thirty-eight days without any syphilitic lesion developing, and then died of bronchopneumonia. Although forty-nine days is the longest incubation period in our experiments, it has only in very rare instances been longer than thirty-eight days. But in consequence of the early death of the animal, this experiment was inconclusive.

A baboon, inoculated with the same mixture as the chimpanzee, is still alive. Six months have elapsed, and no sign of any primary lesion has been seen at either of the sites of inoculation over each orbital arch. But the control animal, another baboon, inoculated with the same virus previously mixed *in vitro* with its own serum instead of that from an immunised animal, did show undoubted syphilitic lesions over each orbital arch.

In another series of experiments we made inoculations at first with simple virus, and then rubbed into the inoculated parts dried and powdered serum from immunised monkeys. We thought that under these conditions the mixing of serum and virus would take place within the tissues. (1) A chimpanzee was inoculated over each eyebrow with virus from a human chancre; forty-five minutes later the inoculated parts were dusted with the fine powdered residue from 9 cc. of the same baboon blood-serum which had already been employed for the chimpanzee in the previous set of experiments (p. 123). The chimpanzee treated with powdered serum died forty days afterwards without any symptom of syphilis; but a control chimpanzee, inoculated with the same virus and then treated with simple dried serum from a baboon which had not been immunised, had a typical chancre on the twenty-eighth day, followed by enlargement of glands behind the jaw. (2) A baboon (*C. sphinx*) was inoculated over each eyebrow with human virus. An hour later the parts were covered with powdered dried blood-serum from two baboons which had been immunised with syphilitic blood. A hundred and forty-three days elapsed without the slightest syphilitic manifestation. (3) On the other hand, a chimpanzee, inoculated on the penis with human virus and treated an hour afterwards with the same powder, contracted a typical chancre at the end of twenty-six days.

The preventive effect of serum (from immunised animals), whether mixed with the virus before inoculation or applied to the site of inoculation a short time later, is by no means constant. In one of our experiments, a macaque (*M. cynomolgus*) was inoculated with a mixture of human virus and serum from a baboon (*C. hamadryas*); a characteristic lesion developed in twenty-four days. But this baboon had itself had a chancre on the brow a year before, and over a period of five months had received 92 cc. of blood from patients with syphilitic roseola. The blood from which the protective serum was prepared was drawn nineteen days after the last injection of human blood. But in spite of these conditions, which appeared to favour the preparation of an active serum, the preventive effect was *nil.*

The facts here summarised clearly show that for prophylactic purposes we need much more powerful forms of serum than those at present obtained. But as some specimens of serum have had a distinct effect, further attempts ought to be made in this direction.

On the supposition that subcutaneous injections of syphilitic virus were insufficient to produce an active serum we set to work to prepare other animals by intravenous injections. But we are not yet in a position to publish anything on the subject.

We selected monkeys for the preparation of serum, not only because these animals can contract syphilis, but also because, from their affinity to man, their serum has only a slight hæmolytic effect on human red blood corpuscles.

Professor Neisser has already made observations on man on the supposition that blood from syphilitic patients might have some preventive effect, but his results were negative. Attempts at preventive treatment by serum from syphilitic patients by Finger and Landsteiner (*loc. cit. supra*) were also unsuccessful. The fact that material from tertiary lesions does not, as a rule, give rise to syphilis when inoculated, might suggest that a gumma contains some substance of the nature of a vaccine. In addition to clinical observations, numerous experiments have been made on monkeys on the inoculability of tertiary syphilis. Salmon made some investigations at the Pasteur Institute, with a negative result. Finger and Landsteiner, however, by using a mixture of the broken-down gummatous material and scrapings from the infiltrated edge of the gumma, did obtain a positive result on two of the lower monkeys.

We ourselves inoculated a chimpanzee over each eyebrow with scrapings from a gummatous ulcer at the root of the nose of a syphilitic woman. In spite of the susceptibility of the chimpanzee, this animal showed no result at the end of thirty-seven days. After this we inoculated the animal at the same sites with virus from the chancre of another chimpanzee, previously mixed *in vitro* with gummatous material from the arm of a man. Neither the original inoculation of tertiary products nor the addition of gummatous material to the virus had the slightest preventive effect, and thirty days after the second inoculation (sixty-eight after the first) a perfectly typical primary lesion developed.

Gummatous material, therefore, contains no more vaccine than virus which has been rendered innocuous by heat. As in our former experiments (p. 108), we made use of virus heated to 51° C. We tried whether better results could be obtained by heating it only to 48° C. The virus thus treated was inoculated over the left eyebrow of a fresh chimpanzee. The absence of any symptom for sixty-three days showed that the virus had been destroyed by heat. After this lapse of time we inoculated the monkey over each eyebrow with virus from primary sores of two macaques (*M. cynomolgus*). The previous inoculation of heated virus had not acted as a vaccine, for a typical primary lesion appeared at the end of thirty days. The chancre of the chimpanzee was much larger than those in the macaques which furnished the virus. We do not attach any significance (from the vaccine point of view) to the fact that though both sides were inoculated, the chancre developed on the right side, the heated virus having been inoculated on the left.

There are, therefore, many difficulties still to be overcome in connection with the prevention of syphilis by the aid of serum or of virus which has been modified by heat. Consequently we have made some further studies of methods which seemed likely to destroy the activity of virus already introduced into the body. Since the syphilitic virus is so unstable that mere warming *in vitro* to 48° C. is sufficient to render it innocuous, we thought that prolonged heating of the site of inoculation might destroy the virus after its introduction. Accordingly we inoculated a baboon (*C. sphinx*) on the penis with virus from hard chancres of two patients. An hour later we dipped the penis of the animal in hot water the temperature of which was gradually raised to 48° C., and kept at that temperature for forty minutes. This did not prevent the development of two small syphilitic indurations on the penis. It is true that the lesions were very small and quickly healed up, but the experiment demonstrated that local heating, under the conditions described, was inefficacious.

Application of a solution of corrosive sublimate had no greater effect. A macaque (*M. cynomolgus*) was inoculated over each eyebrow with virus from a human hard chancre. An hour later the places were washed for four minutes with a 1 per cent. *solution of corrosive sublimate*, but after an incubation period of twenty-three days the macaque presented suspicious lesions at the sites of inoculation which quickly got well. As in the preceding experiment the primary lesion was very slight, but the treatment was not capable of completely preventing it.

We also employed *mercurial inunction* in several experiments with the object of destroying the virus after inoculation. A chimpanzee was inoculated over the eyebrow with virus from hard chancres of two male patients. Three-quarters of an hour after inoculation the parts were rubbed for ten minutes with strong mercurial ointment (equal parts of mercury and benzoated lard). The application was followed by irritation and local inflammation on the left side, where a crust formed, which then fell off after a short time. No syphilitic lesion of any kind developed on either side. This negative result was not due to inactivity of the virus, for a control chimpanzee inoculated with the same virus at the same sites developed typical chancres on each side after an incubation period of twenty-eight days. Nor could it be attributed to the local irritation, for there was no chancre on the right side, which was not in the least inflamed by the inunction. The short interval between the inunction and the formation of the crust on the left side, as well as the appearance of the lesion, and the absence of glandular enlargement, are sufficient to show that the lesion was not syphilitic. And to render this still more certain we made a second inoculation of human virus in the same chimpanzee on the forty-ninth day from the first. The virus was taken from hard chancres on the penis and lower lip of two patients, and inoculated over each eyebrow and on the penis. A typical chancre developed over the left eyebrow thirty days later (*i.e.*, seventy-nine days after the first inoculation), followed in six days by local glandular enlargement; and two characteristic chancres appeared on the penis nine days after that over the left eyebrow, with subsequent enlargement of the inguinal glands. About a month after the first primary lesion a typical mucous patch appeared on the upper lip.

This experiment demonstrates that the virus of the first inoculation was destroyed by the mercury without conferring the slightest immunity against a subsequent inoculation with syphilitic virus.

A similar experiment was made on a macaque (*M. cynomolgus*). It was inoculated over both eyebrows with human virus, and an hour later the places were rubbed with the same mercurial ointment. The next day small crusts formed, but no syphilitic lesion occurred.

Mercurial inunction, then, is capable of preventing syphilitic manifestations when applied an hour after inoculation, but its irritating effect is inconvenient. We therefore sought for other preparations, and tried the *local application of calomel.* A chimpanzee was inoculated over each eyebrow with virus from the hard chancres of two patients. An hour and three-quarters later the places were rubbed for five minutes with calomel ointment (one part of calomel to two of lanolin). The animal died thirty-two days later from broncho-pneumonia without any syphilitic lesion. A macaque (*M. cynomolgus*) treated in exactly the same way lived for 110 days without any local lesions—neither irritation from the ointment nor any primary lesion. A baboon (*C. sphinx*) was inoculated over each eyebrow with virus from hard chancres of two patients. An hour later the places were rubbed with calomel ointment. The next day the parts were red and the lines of scarification very distinct, but no inflammation followed and the places quickly healed. No primary lesion had occurred at the end of two months, while a control animal inoculated with the same virus but not anointed showed a typical chancre over the left eyebrow after an incubation period of forty days. Two months later the monkey was once more inoculated over both eyebrows with human virus, and twenty-four days later two red spots appeared which soon developed into typical chancres. This test inoculation supports the view that calomel ointment impeded the action of the virus at the first inoculation.

In another experiment three macaques (*M. cynomolgus*) were inoculated over the eyebrows with virus from two human hard chancres. One was kept as a "control," whilst the two others were treated locally with calomel inunction an hour after the inoculation. The control animal had a typical hard chancre on each side twenty-eight days after inoculation, while the two others were free from any lesion at the end of sixty-eight days. The calomel ointment did not excite the slightest local inflammation.

Having proved that local treatment of parts contaminated by syphilis can hinder the development of the disease, it was important to determine how long the virus may remain localised at the site of its introduction. To clear up this question we inoculated a macaque (*M. cynomolgus*) on the tip of the ear with virus from a human hard chancre. Twenty-four hours later we removed the upper part of the ear. Sixty days after inoculation, as the animal had shown no symptom of syphilis, we made fresh inoculations over the eyebrow, and typical chancres developed on each side. It follows, therefore, that the syphilitic virus had remained localised for at least twenty-four hours. A chimpanzee and a baboon also inoculated in the ear to test the limit of time during which the virus does not become localised died before the end of the experiment, so that the question can only be settled after further experiment.

III.—THE MICRO-ORGANISM OF SYPHILIS.

After repeated failures, the search for the micro-organism of syphilis has at length been crowned with success. The organism, a very slender mobile spirillum, was discovered by Schaudinn in matter from syphilitic lesions, and more completely investigated jointly by Schaudinn and E. Hoffmann[5] [6]. Schaudinn at first named it *Spirochæta pallida*, but now prefers the term *Spironema pallidum*, as proposed by Vuillemin.[7] This change of nomenclature was suggested by the numerous points of difference between the organism of syphilis and the recognised forms of *spirochæta* such as *S. refringens* and *S. plicatilis*. The first is characterised by the presence of vibratile cilia, by its thickness as well as by the number and configuration of its spirals. Schaudinn felt obliged to emphasise the distinctive characteristics of the syphilitic spirillum, in order to avoid errors which might be caused by confusion with spirilla somewhat similar in appearance. Already some observers have believed they had found the same spirillum not only in syphilis but in other diseases. Thus Kiolomenoglou and Cube[8] affirmed that the spirillum of Schaudinn was present in a sloughing carcinoma, in tuberculous abscesses, in gonorrhœal pus, &c. Hoffmann himself found a very similar organism in a non-syphilitic case.

In prosecuting his researches on spirilla, Schaudinn ultimately differentiated four or five species (in addition to the syphilitic *Spironema*) in morbid tissues and fluids of human patients. He found several different spirilla in the preparations of Kiolomenoglou and Cube in cases where these observers had thought that the spirillum of syphilis was present. All these delicate investigations were undertaken to settle the question as to whether in cases of syphilis there was an organism perfectly distinct from allied species. Up to the present no one has succeeded in cultivating the spirillum, so that its recognition can only be effected by observing its morphological characteristics under the microscope. But identification by such means is regarded with some scepticism after what took place in connection with the supposed morphological characteristics of the tubercle bacillus, the cholera vibrio, &c. At one time it was thought that it was sufficient to measure a bacillus, and to stain it in a certain way in order to identify it with certainty. But at the present time such confidence is no longer entertained. So that, *à priori*, it is not surprising that a spirillum should be found in non-syphilitic cases which, so far as microscopic appearances go, closely resembles the *Spironema pallidum*, just as in syphilis itself other spirilla may be found scarcely to be distinguished from it. Even the way in which it takes certain stains, which Schaudinn considers sufficient to differentiate it, is not absolutely constant. According to him the syphilitic spirillum, stained by Giemsa's method, should take on a pale pink tint, whilst other analogous spirilla are stained a bluish colour. But in the course of our investigations, in a primary sore of a macaque we met with typical pale, slender spirilla which were stained pale blue by Giemsa's method. This primary sore when inoculated in a chimpanzee gave rise to a chancre, and in this case the spirilla, exactly similar in all other respects, were stained pale pink by Giemsa's stain, taken from the same bottle. Observers must be careful not to attribute too much importance to small details, such as shades of colour, the number of turns in the spirals, &c.

The most important facts are: (1) That Schaudinn's spirilla can almost always be demonstrated in primary and secondary lesions both in man and monkeys; (2) that these spirilla have been demonstrated in cases of syphilis in both hemispheres (Europe and America); (3) that they are present, often in large numbers, in congenital syphilitic lesions in the new-born; (4) that their presence has been demonstrated in the blood by several observers, notably by Noeggerath and Staehelin.

These facts, even in the absence of cultures, suffice, in our opinion, to establish the etiological *rôle* of this organism. No secondary organism has been found under these several conditions. Moreover, one is obliged to recognise trypanosomes as the etiological agent in various diseases in spite of the failure to obtain cultures of them. We hold, therefore, that syphilis may fairly be regarded as a chronic relapsing spirillosis. Supposing that, in a non-syphilitic case, one were to find a spirillum with ten to twenty-four narrow turns, staining pink with Giemsa's stain, and presenting several other characters of *Spironema pallidum*, that would in no way disprove the etiological importance of this organism in syphilis, but would only prove that several spirilla exist with similar microscopic appearances, just as the tubercle bacillus and the bacillus of leprosy, the vibrio of cholera and the vibrio of Gamaleia, are surprisingly alike when examined in stained films under the microscope.

Since the discovery of Schaudinn's spirilla in syphilitic lesions of monkeys forms one of the important arguments in favour of the etiological part played by this organism, it will be useful for us to complete the account of our work which was given in part in an earlier communication.[1]

When this was published we had demonstrated the presence of pale spirilla in four monkeys, a chimpanzee, a baboon, and two macaques. In another chimpanzee, whose chancre was healing when examined, and in a macacque, we had obtained a negative result.

Up to now we have looked for the syphilitic spirillum in thirty-one cases. In chimpanzees we have found it eight times out of ten. One of these negative cases has been quoted above. In the second we searched for the spirillum on two occasions, but the death of the animal shortly after the appearance of the chancre put a stop to our investigation. This negative result was unimportant, since a second chimpanzee, inoculated with the same human virus at the same time and in the same sites, furnished abundant spirilla on the second day, as numerous as in congenital syphilitic lesions of the new-born, and occasionally grouped into clumps. A month later the chancre still contained abundant spirilla, and the same organisms were found in cover-glass smears from a mucous patch in its mouth, mixed with other organisms, mostly cocci. In secondary papules on the skin in this animal we failed to demonstrate the presence of spirilla.

In one of our chimpanzees, inoculated on the penis and over the eyebrow, the chancre in the latter position was rich in pale spirilla, while we were unable to find a single specimen in that on the penis. This observation, along with many others, shows great variability in the distribution of syphilitic spirilla. For instance, in examining, at intervals of a few days, the exudation from the same chancre of the same monkey, we sometimes found pale spirilla in abundance, and at other times none at all.

Out of six baboons (*C. sphinx*) we found the pale spirillum in four. Of the other two, one had been inoculated with serum from a human chancre, and the second with scrapings from a secondary cutaneous papule of a chimpanzee, and both had typical primary sores.

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[1] See *Bulletin de l'Acad. Méd.* 1905, vol. liii., p. 468.

Out of fifteen macaques we were successful in eleven. In one macaque (*M. cynomolgus*), inoculated from the chancre of the chimpanzee which yielded such abundant spirilla, a typical chancre developed, but we could not find any spirilla. A second macaque of the same kind was inoculated with scrapings from a mucous patch of the same chimpanzee, but although the scraping was rich in spirilla, we could not find one in the typical hard chancre which resulted. A third (*M. cynomolgus*), inoculated from a human chancre, and a fourth (*M. sinicus*) have given negative results. But we ought to state that in each of these two cases we made only four preparations with Giemsa's stain. But several times, even in our positive examples, we have only found very scarce spirilla in one preparation out of four. Possibly if we had examined five or ten preparations the result in these two cases might have been positive. We did not go so far as that, because our conviction of the etiological importance of Schaudinn's spirillum was so strong that we did not think it worth while to fatigue our eyes for the sake of seeing the organism once more. Besides, one knows that in chronic affections due to organisms, such as lupus, one may fail to find the bacillus even in extensive lesions of the skin.

To sum up, we found the spirillum in twenty-three out of thirty-one monkeys examined, or 74 per cent. This result gives an explanation of the failures experienced formerly by ourselves and by our predecessors, Bordet and Gengou. If it is borne in mind that, in spite of perfected methods and practice in looking for the spirilla, we failed in 26 per cent. of the cases to come across the spirilla, a ready answer is found for C. Fraenkel,[9] who enquires how it is that the pale spirillum was never found before Schaudinn's researches. Even in syphilitic lesions of the new-born, while there are cases where the spirilla are abundant, there are others in which they are very scarce, and in which it may not be possible to demonstrate any.

In our investigations on the syphilitic spirillum in animals, we have never found the *spirochæta refringens*, nor any other spirillum except that of syphilis.

In conclusion, we wish to express our thanks to Dr. Salmon, who has assisted us from the commencement of our investigations, and to Dr. Weinberg, who has made the *post-mortem* examination of most of our chimpanzees.

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SELECTED ESSAYS

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10

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WITH FIVE FIGURES IN THE TEXT

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THERE is scarcely a mollusc or an earthworm that fails to reveal one or more internal parasites belonging to the class of parasitic protozoa known as the Sporozoa, while crustacea, insects, and lower vertebrates are commonly infested by them. They have been found in hosts belonging to protozoa, cœlenterata, platyhelmia, nemathelmia, nemertina, chætognatha, rotifera, polychæta, oligochæta, hirudinea, gephyrea, polyzoa, crustacea, onychophora, myriapoda, hexapoda, arachnida, mollusca, echinodermata, enteropneusta, tunicata, cephalochorda, pisces, amphibia, reptilia, aves, and mammalia. With the apparent exception of the sponges, therefore, there is no phylum of the animal kingdom that is not liable to sporozoon infection, and, in view of this wide distribution, the surprising thing would be not that man should be infected, but that he should escape their ravages. With the growth of our knowledge concerning the sporozoa, it has been found that human organs and tissues, like those of all other animals, may indeed be the seat of certain types of these organisms. Thus, *Coccidium cuniculi* (?) has been found in the human liver; *C. perforans* and *C. bigeminum* in the intestine; *C. oviforme* (?) in the brain; "*Eimeria*" *hominis* in the pleural cavities; *Coccidioides immitis* in the skin; *Laverania malariæ, Plasmodium malariæ,* and *P. vivax* in the blood, and *Sarcocystis lindemanni* and *S. immitis* in the muscles and liver. The specific nature of many of these has been contested, but some, *e.g.*, the malaria-causing organisms, have been established beyond question, and without Koch's final test of pure cultures and inoculations from them.

Among the contested forms must be included the so-called "vaccine bodies," which have been regarded by some observers as protozoan organisms, by others as degeneration structures or by-products of the cell, ever since Guarnieri, in 1892, giving them the specific name *Cytoryctes vacciniæ*, made the first experimental test to demonstrate their nature. Equal credit, however, should be given to von Wasielewski (1901), whose painstaking experiments and logical deductions left no reasonable doubt as to the nature of these inclusions in vaccinated tissues.

In every sporozoon of which the life-history is known, there are definite reproductive phases characteristic, perhaps, of some phase of the disease, or of some peculiar environment of the parasite. Thus, in the malaria-causing organisms the asexual reproduction, or schizogony, takes place in the blood corpuscles of man or birds, and the sexual cycle, or sporogony, takes place in the digestive tract of the mosquito. In all probability an analogous series of processes takes place in the parasite causing vaccinia and variola. The phase of the organism known as the "vaccine body," with which Guarnieri and Wasielewski experimented, is found in variola as one stage of the life-cycle. A second and more important cycle takes place within the nuclei of the generative cells of the skin, and includes all of the processes of propagative reproduction. As the discovery of the mosquito phase in malaria gave the final proof in this disease, so here the discovery by Councilman, Magrath, and Brinckerhoff, of the intranuclear phase in cytoryctes, gave the key to the life-history of this sporozoon.

It has been my great privilege to work with Dr. Councilman upon material prepared for his own use, and it is owing to his generosity in sharing this material with me that I am able to offer the following interpretation of the life-cycle of *Cytoryctes variolæ*.

Efforts to cultivate sporozoa on artificial media and thus to get pure cultures have never succeeded, and it is impossible, therefore, in our present state of knowledge, to apply Koch's test to prove that we have to do with an organism. Furthermore, owing to the nature of the subject, there is but a limited range of experiments possible, and the stages in the life-cycle must be worked out from morphology alone, and without the advantage of the living organism. Dependent thus upon fixed material, there are obviously many possible sources of error, and we cannot hope to fill in every gap, or to avoid errors of interpretation. In the first place, the material upon which the study is based could not be absolutely fresh when fixed, and the time which usually elapses between the death of the patient and the autopsy was much too long to justify the hope of obtaining perfectly normal protozoan structures. Only a student of these delicate unicellular organisms can appreciate the absolute necessity of obtaining protozoa directly from their normal environment, and I do not doubt that many of the bizarre structures which we have observed are due to this technical, but unavoidable, error. In the second place, Cytoryctes belongs to the group of microsporidia, and here, as in the allied myxosporidia, the complete life-history of no species is known, the sexual cycle being entirely unrecognised at the present time. Analogies for many phases of Cytoryctes must, therefore, be drawn from groups as distinctly related to it as the coccidiida and the gregarinida. In the third place, the morphological method involves the possibility of confusing artefacts due to technical methods, and degeneration products of the tissue cells, with stages of the organism. I have had this possibility of error constantly before me during my work, and freely confess that, owing to the extremely small size of certain stages and absence of differentiation, I cannot always distinguish them from artefacts. The spores, for example, even with their great refringency and conspicuous points (which are characteristic of all microsporidia spores), cannot always be distinguished from minute bits of matter of one kind or another. The difficulty vanishes only when these minute stages of the organism are accompanied by developmental phases which are always unmistakable.

The one factor which, more than morphology or staining reaction, has guided me in my interpretation of the life-cycle, and has enabled me to distinguish between the minute phases and artefacts, is the fact that the growth stages are always present in sequence, a sequence which follows *pari passu* with the development of the skin lesions.

Although the microsporidia and the myxosporidia are so little known, and the published observations upon them so inadequate, they may serve as a basis for comparison with the complicated life-cycle of the small-pox organism.

The genus Thèlohania (Henneguy) as worked out by Thèlohan (1895), and more recently by Stempel (1902), begins as a minute uninucleate, spherical fragment (interpreted by Thèlohan as a " pansporoblast "). The nucleus forms a primitive mitotic figure, the cell thus dividing by indirect division; two daughter nuclei repeat the division; the four resulting nuclei again divide, and eight " sporoblasts " are finally formed. Each of these then changes into a single pyriform spore. Like the intranuclear phases of Cytoryctes, all stages of Thèlohania are vesicular.

In Plistophora (Gurley), which is also vesicular, the spheres are larger, and include a larger number of sporoblasts, each of which again forms a single spore.

The genus Nosema (Nägeli), because of its importance in the silk industry, is among the best known of the microsporidia. One species, *N. anomalum*, infests the stickleback; *N. destruens*, a marine fish, Calionymous lyra; another, *N. ovoideum*, the liver of Cepola rubisens and of Motella tricerata, which are also marine fishes; still another, *N. bryozoides*, infests the tissues of the bryozoön Alcyonella fungosa; *N. lophii* attacks the skate, and one other, finally, and the most important of all, *Nosema bombyces*, causes the famous pèbrine disease of silk-worms. In the latter case the parasites invade all of the tissues and organs of the caterpillars. The valves of the spores (which are taken in through the digestive tract) open and liberate an amœboid germ, which, like the zygote of the malaria organism, bores into or through the gut epithelium, and completes its development in the body cavity. It becomes multinuclear, each nucleus giving rise to a "pansporoblast," which, in turn, gives rise to many spores. Sex-differentiation and conjugation are absolutely unknown.

In Cytoryctes variolæ the life cycle is much more complicated. The first development of the germ in the host is unknown ; it probably takes place in the seat of primary infection, forming an organism which reproduces by germs, probably similar to those which I have named "gemmules," the process being known as "multiplicative reproduction." The gemmules are probably carried in the blood to the skin, where the further development takes place. This early part of the development, therefore, is purely conjectural, but from this point the observations are fairly complete. The gemmules become intracellular (cytoplasmic), amœboid organisms which give rise to similar gemmules. This process, which Councilman has designated the "vaccine cycle," must continue for some time, for in variola the gemmules are distributed to all regions of the skin. Ultimately the germs derived in this way give rise to forms which penetrate the nuclear membrane, and develop into gametocytes (?) of two types, one forming the supposed male gametes, the other the female. The gametes conjugate (?), the zygote thus formed developes into a comparatively large, amœboid organism, in which the pansporoblasts originate. These pansporoblasts give rise to primary sporoblasts, and the latter to multitudinous spores, the entire process taking place within the nucleus, and corresponding to the so-called "propagative reproduction" of other sporozoa. The spores thus formed may, in turn, infect fresh nuclei, and grow directly into new secondary sporoblasts, which give rise to similar spores, a true "schizogony," and a second means of auto-infection, by which the organism spreads throughout the nuclei and cells of the skin, and possibly to many of the other organs of the body, like the allied Nosema bombyces, which infects every tissue and cavity of its insect host. These spores finally may transmit the disease to new hosts.

A.—Methods Employed.

The material used in the study of Cytoryctes was all obtained from human skin taken at the time of the autopsy.

It was fixed in Zenker's fluid, hardened and kept in alcohol, embedded in paraffin, and cut into as thin sections as the tough nature of the tissue would allow. The stains used were the iron hematoxylin of Heidenhain, Mallory's chloride of iron hematoxylin, the polychrome methylene-blue-eosin combinations (Weigert and Romanowsky), and the Borrel stain.

The use of different stains on small-pox tissue shows that the organism is composed entirely of material which colours like the chromatin of tissue nuclei. The more delicate differential stains, such as the methylene-blue-eosin compounds, or the Borrel, show, however, that, although it is like chromatin, there is differentiation, and two grades, differing apparently in the degree of nucleinic acid present, compose the bodies of these minute cells. The iron hematoxylin stain, either alone or with counter-stains, gives no such differentiation, and all stages stain as do the nuclei of the tissue cells, although some stain more intensely than does the chromatin of these nuclei. Iron hematoxylin, therefore, is not a satisfactory stain for tracing out the stages of the life-history.

The Romanowsky stain, used in a modified form for sections, gives beautiful results for certain phases. It is prepared from the directions given by Unna; the commercial polychrome methylene blue of Grübler is used full strength, and to this is added enough of a two-tenths per cent. aqueous solution of eosin to form an insoluble precipitate on the surface, and after this is removed with filter paper the mixture is ready for use. The sections are run through successive grades of alcohol (the careless method of plunging directly from 95 per cent. into water is to be avoided) to water, and when completely hydrated they are immersed in the mixed stain and left for about thirty minutes. At the end of this period they are thoroughly rinsed in water, and dehydrated by passing again through the successive grades of alcohol. Differentiation should take place in the higher grades (95 per cent. or absolute alcohol), and the sections should be cleared in xylol and mounted in balsam. Preparations made in this way are not always successful, the result frequently being too pale, or the differentiation imperfect, but with care good results follow in the majority of cases.

When successful, the cytoplasmic phases of Cytoryctes are blue, with an occasional deeper blue central point, while those in the nucleus are either blue or pink. If in the earlier stages of sporulation, the entire organism is blue like the chromatin, and if in the later stages only the spores and chromatin are blue, the residual bulk of the organism staining pink. This stain is very important for clearly defining the spores in the nuclei and for the early development of such spores. In the earlier stages of the pansporoblast, also, it has certain advantages, but is of no value in the later stages. It is also of great importance, when used in connection with the Borrel stain, in differentiating streptococcus.

The other combination of polychrome methylene blue and eosin (Weigert) is particularly good for the developmental stages of the primary and secondary sporoblasts, the early stages of spore-formation, and the differentiation of the cytoplasmic organism, but not so good for pansporoblast stages. The spores are also brought out very clearly by this stain.

The Borrel stain (see Borrel, 1901) consists of indigo-carmine and picric acid, used after a preliminary staining with magenta in saturated aqueous solution. The picro-carmine combination is made by adding one part of a saturated aqueous solution of picric acid to two parts of saturated indigo-carmine solution. The sections are hydrated as for the Romanowsky stain and immersed for about twenty minutes in the magenta solution; they are then rinsed with water and immersed in the picro-carmine for about five minutes. Differentiation takes place in the alcohols. Borrel, in using this stain, obtained a brilliant red coloration of the tissue nuclei and of the cell inclusions (in carcinoma), but for variola material I find that the best results are obtained by extracting all of the red from the tissue nuclei, leaving the chromatin and cell bodies green and the connective tissue blue, while the organism, in all its phases, stands out a brilliant red upon this striking background. The entire organism does not stain with the red, and many stages of its development indicate a differentiation shown by red and green colours. Thus the early cytoplasmic form stains a homogeneous red, but with growth the green appears, and all stages of the further development are marked by the same differentiation. The nuclear form is usually red, but in the later period of sporulation the red spores lie in a green matrix, the same colour as the chromatin of the tissue nuclei. The pansporoblast stages are also clearly demonstrated by the use of this stain. The developing sporoblasts are red, lying in the dense, homogeneous, green-coloured plasm of the pansporoblast, which is distinguished from the cytoplasm of the cell by its form and its greater density. I cannot recommend this stain too highly for work of this nature, it has been my main reliance in working out the different stages of cytoryctes, but it must be clearly understood that it is in no sense selective for the small-pox organism, and cannot always be depended upon to distinguish the parasite from chromatin.

B.—PHASES OF THE LIFE-CYCLE.

The forms to which the small-pox organism are most closely allied, and which are usually included under the name myxosporidia, cover a wide range in habitat, and have widely varied structures. As a rule they have certain definite points in organisation and in development that bespeak a taxonomic unity and justify the grouping of many apparently diverse types under one heading. Of all sporozoa they bring about the most deleterious effects upon the cells and tissues of the invaded host, and, more than any other group of the protozoa, are the cause of the most virulent epidemics. Up to the present time no epidemics among men have been traced to them, but among the lower animals, especially the lower vertebrates and arthropoda, most destructive epidemics are caused by them. The organs attacked are many, and differ with the species. In recent classification systems all of these malignant forms are grouped together under the name of neosporidia, and these are divided again into two orders, myxosporidia and microsporidia, which are distinguished, in a general way, by the fact that the former are intercellular parasites, the latter intracellular. This distinction, however, is somewhat arbitrary and has little value in taxonomy. Connective tissue and muscles are the more common seats of infection for all of these parasites, but they may occur in any tissue which is soft enough to be penetrated. Any specific form may attack a given tissue or organ, or it may spread over the entire host-organism, as in the pèbrine disease of silkworms. In the concentrated types a cyst is usually formed, within which the bulk of the parasites are contained, but in the other type, which gives rise to the condition termed "diffuse infiltration" by Thèlohan, the parasites are spread over comparatively large areas of the infected tissue. The cysts of the concentrated types may be seen by the unaided eye, but the diffuse types form no visible structures. It is the latter that cause the most severe epidemics in animals, and it is to this type that Cytoryctes, the cause of small-pox, belongs.

(1) *The Early Cytoplasmic Stages.*—(*a*) *The Youngest Forms.*—Cytoryctes variolæ is first seen in the skin in the form of a cytoplasmic inclusion of minute size. Like the inclusions in vaccinia, they are spherical, homogenous bodies which gradually become differentiated as growth proceeds. The evidence of every stage in this cycle indicates that in vaccinia and variola we have to do with the same organism which, in vaccinia, has undergone some modification by reason of which the nuclear phase is inhibited. The cause of the functional difference, like the resulting immunity in the host, remains unexplained.

The early cytoplasmic form in variola is interpreted as a gemmule coming from the sporulation (?) ("multiplicative reproduction"?) of the organisms of the primary infection. The smallest that I have seen measures seven-tenths of a micron, and in this earliest stage there is no evidence of structure, the minute spheres appearing perfectly homogeneous with every stain used. It may be alone in the cell or there may be several of them in different stages of development. All efforts to detect a central body which might be interpreted as a nucleus have been futile; like a spermatozoon within an egg, the organism is all nucleus, as it were, and differentiation into nuclear and cytoplasmic material comes later.

(*b*) *Growth and Differentiation.*—Growth of the parasite can be followed stage by stage from a centre of development in the skin. Thus, a field under the microscope, in which the cells contain the youngest germs, may be regarded as a centre, while adjacent fields contain older growth stages arranged in regular sequence in respect to that centre. A field next to that containing the youngest forms will not contain adult amœboid organisms, but a developing series of growing forms; consequently it is comparatively easy to trace the continuous development of the germ into the adult organism. One of the first indications of differentiation is vacuolisation in the centre and the occasional appearance of a minute central dot, which with methylene blue stains more intensely than the remainder of the body, but with the Borrel is not so clearly differentiated, appearing as a central aggregate of the same material as that forming the periphery. At this stage the organism measures about three microns. Differentiation in the peripheral portion is first indicated in the growing organism by the appearance of minute, unstained spots. These become larger and then take the green stain of the Borrel combination, the rest of the periphery taking the red. From this point on the Borrel stain indicates a clearly-marked difference in the chemical make-up of the young organism. Like the microgametes of the malaria organism (see fig. 4, p. 168), comparatively large masses of the body stain red, while the general basis is green, a differentiation which is retained throughout. The red staining material forms the substance of the gemmules which appear at a later stage, and is to be interpreted as the germ-forming material, to which I shall give the provisional name *protogonoplasm.*[1]

During these growth stages the form assumed varies widely; often it is spherical, but may be fusiform, pyriform, or amœboid, while pseudopodia are frequently caught in various degrees of extension. Whatever the form assumed the organism always lies in a vacuole which marks the limit of its range in the cell. A favourite position appears to be the immediate vicinity of the nucleus of the epithelial cells, and it is frequently found closely encircling the nuclear membrane. I have not enough evidence to warrant the belief that the organism, in the adult stage, can migrate from cell to cell, although in some cases where the cell boundaries are broken down by degeneration there is an indication that such migration may take place.

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[1] *πρωτος* = first ; *Υόνος* = seed ; *πλάσμα* = anything formed.

(*c*) *The Adult Amœboid Organism and Gemmule-formation.*—In general structure the protoplasm of the cytoplasmic form is very simple. It is finely granular, and, apart from the chromatin-like granules of protogonoplasm, is without inclusions. There are no vacuoles and there is no differentiation into ectoplasm and endoplasm. There is no nucleus, strictly speaking, the only approach to such an organoid is the occasional, more intensely staining dot in the early cytoplasmic form. No mitotic figure, like that described by Thèlohan in the genus which bears his name is present at any period, and the only appearance of a division phase is an occasional elongate granule, which has some resemblance to the "Hantelform" of the nucleus in some species of Nosema (Doflein, 1898). The entire condition of the nucleus in Cytoryctes is identical with that in *Lymphosporidium truttæ* (*cf.* Calkins, 1898), where the spores are likewise composed of similarly aggregated granules.

One prolific method of auto-infection in variola is by the "multiplicative" process of reproduction, by which the mature amœboid organisms give rise to great numbers of reproductive bodies which I have called the gemmules, to distinguish them from spores and sporozoites of other forms of sporozoa.

In the largest forms of the cytoplasmic parasite (measuring from 10 to 14 microns), the red-staining protogonoplasm is usually in some stage of gemmule-formation. In many cases it is distributed throughout the body of the organism in the form of minute spherical granules, which become more and more definite in the later stages of growth, until, when practically mature, each of them lies in a minute vesicle. These granules are demonstrated by almost every chromatin stain that has been used, and the later spore stages may be made out with the iron hematoxylin. The conditions in gemmule-formation were first clearly indicated by the Borrel preparations in which the gemmules stain red, while in addition there is a red framework, or network, which extends throughout the entire body. This is composed of very fine granules, and the accumulation of these into larger groups gives the appearance of masses similar to the gemmules. With the general diffuse chromatin stains, like iron hematoxylin, these are difficult to distinguish from the reproductive bodies, but with the more delicate stains the nature of the framework can be clearly made out.

Nothing more definite can be stated in regard to the formation of the gemmules, although the process is probably similar to that which takes place in other enucleate organisms, as, for example, in *Bacillus bütschlii* (Schaudinn, 1902; see fig. 3, p. 163), or spore-formation in *Lymphosporidium truttæ* (Calkins, 1898). The protogonoplasm in later adult stages is granular and diffused throughout the body, and the gemmules are formed by the accumulation of these granules in the same way as in the parasite of the brook trout. However obscure the details of gemmule-formation are, there is no doubt whatever that the material composing the gemmules and that of the diffused protogonoplasm is one and the same substance. The gemmules in the mother-organism are, apparently, not always identical in size, measuring from $\frac{7}{10}$ to 1 micron, but they correspond to the smallest germs observed in the cytoplasmic cycle, and the differences are well within the range of individual variations of spores in general. In one instance they were found emerging from the parent cell, some being outside, while others were within. The gemmules are liberated by the disintegration of the framework, which remains as a residual structure in the host-cell.

(*d*) *The Cytoplasmic Residuum.*—This residual framework is of interest, for it persists in the cells of the skin for some time after the reproductive bodies are distributed, and may assume various fantastic forms. It is, therefore, of the nature of residual structures found in all forms of sporozoa, and described under different names (*Sporenrest*, *Restkörperchen*, *Reliquat de différéntiation*, *&c.*). An apparently similar "stringy" residual structure is found in the sarcosporidia. "Between the pansporoblasts (?) there remain strings of protoplasm, which together form a network, or a system of chambers left over after the spores are gone" (Doflein, 1901, p. 215). There are no figures in Doflein's work to illustrate this structure, and the staining reactions are not given, but the description applies very well to the residual structure of the cytoplasmic phase of Cytoryctes. Here there is always a more or less definite meshwork, which becomes much distorted, until finally it becomes a mass of disarranged fibrils, still retaining, however, the characteristic staining reactions.

(2) *The Early Intranuclear Phase.*—(*a*) *The Gametocytes.*—The germs formed by the multiplicative reproduction of the cytoplasmic amœboid form of the parasite may develop into new cytoplasmic organisms, or, ultimately, may become germ cells within the nucleus. In the latter case they develop into structures which I regard as gametocytes, the products of which probably conjugate. The resulting zygote is the amœboid pansporoblast mother organism. This hypothesis is based upon the following observations:—

In regions of the skin where cytoplasmic reproduction occurs, some cells are flooded with young forms, apparently identical with the young cytoplasmic forms or gemmules. Some of these are seen in the nucleus and some on the nuclear membrane. Again, in regions of the skin where the beginning stages of the cytoplasmic organisms are found, similar minute, clearly defined, homogeneous bodies are found within the nucleus. These intranuclear structures are entirely different from the nucleolus-like chromatin masses and different also from the spores in the chromatin, for there is no vesicle and no refraction (see *infra*). Like the cytoplasmic form they stain uniformly, and a progressive series of sizes can be traced from the point of infection. We also find intranuclear adult forms, apparently in the process of gemmule-formation, but without the characteristic chambered structure of the cytoplasmic forms.

The gemmules formed within the nucleus are very definite in size and shape and are less numerous than those of the cytoplasmic organisms. I am unable conclusively to interpret this process, but think it highly probable that these intranuclear gemmules are connected with the sexual cycle, and that the mother-organism may be the female gametocyte, while the male gametocyte, in its latest phases at least, is quite different. Such a method of female gamete-formation is not without parallel in the sporozoa (see Nusbaum, 1903). Unfortunately, however, there seems to be, as yet, no absolute way in which the supposedly younger nuclear forms of these gametocytes can be distinguished from chromatin of the tissue nuclei; for, although ordinarily green with the Borrel stain, it might be argued that we do not know what chemical changes the chromatin might undergo through the agency of the unknown toxines produced by the cytoplasmic parasites. The intra-nuclear amœboid form, like the cytoplasmic, becomes differentiated into red and green portions, but here again the objection might be raised that transitions between the green and red chromatin are to be expected in degenerating nuclei.

The direct entrance of gemmules into the nucleus from the cytoplasmic adult form, as mentioned above, is highly probable, for broken-down residual structures are occasionally seen with partly developed gemmules, while bodies are seen in the adjacent nuclei which are similar in size and reaction to the gemmules. I am aware of the extremely meagre data upon which to base the assumption that these gemmules ultimately enter the nucleus. The above arguments are supported by the fact that the end of the regular growth sequence of the cytoplasmic form is the beginning of the intranuclear sequence, and this region is the place to look for young sporoblasts. The only conclusive proof for this assertion is to actually see the living gemmule enter the nucleus as Schaudinn has seen the sporozoite of *Plasmodium vivax* enter the human blood corpuscle. Since this is impossible, we must fall back upon the one incontrovertible fact that the pansporoblast mother-organism arises inside of the nucleus, and this being true, there must be some embryonic form in the same place.

In small-pox cases characterised by late cytoplasmic and early nuclear forms, there are frequent groups of spherical organisms in the cytoplasm, which appear like the young intranuclear forms. With methylene blue these stain intensely and appear to be cytoplasmic forms of the intranuclear spherical type. With the Borrel they sometimes stain red, but more often green, showing evidences of degeneration. In groups of gemmules which have remained in the region occupied by their mother-organism, similar "ring-forms" are found in stages varying in size from the gemmule to comparatively large forms. These stages are, I believe, to be interpreted as sexually mature gemmules which have failed to reach the definite nuclear position where their further development is possible. From the fact that older stages are unknown, there is reason to believe that if they remain cytoplasmic they do not develop beyond the ring stage, and ultimately change in colour reaction from red to green. Within a nucleus, however, they retain their affinity for the red of the Borrel combination. Here they form what I regard as the male gametocytes, the female being formed, presumably, in the manner described above. They appear first as homogeneous granules or gemmules within the nucleus, but they early assume the spherical form with central red-staining masses. As they grow the red-staining material remains in the centre, while similar red-staining points, derived, probably, from the central mass of protogonoplasm, develop at the periphery. In the early stages the entire sphere retains the red, but later the central portion and the points in the periphery alone retain the red, while the peripheral matrix stains green. These bright red points are, as I believe, the almost sub-microscopical male reproductive elements. After their liberation the gametocyte framework persists as a residual structure analogous to that of the gemmule-forming cytoplasmic phase. It remains intranuclear, changes in colour reaction from red to green, and ultimately degenerates. The latter process is indicated by the enlargement of the peripheral vesicles, and the change of the central mass into one or more great vacuoles. These frequently unite into a central spherical vesicle, thus forming residual bodies, consisting of one ring within another.

The central bodies of the gametocytes are to be looked upon as "Restkörperchen" analogous to those of all other male gametocytes of the sporozoa. They, together with the central rings of the residual frameworks, serve to distinguish these structures from the sporoblasts.

In the entire sub-class to which Cytoryctes belongs—the neosporidia—no one has observed conjugation, and as Minchen (1903) observes, this fact makes it probable that conjugation takes place between minute forms. In the Cytoryctes cycle no structure has been found which can be interpreted as a fertilisation stage, although the pansporoblast phase, with its high potential of reproduction, would indicate that fertilisation must take place.

In a footnote, Stempell (1902, p. 262) states that Schaudinn has seen the conjugation of two amœboid forms of Nosema bombyces which had just emerged from the spore. We await the publication of Schaudinn's result with interest, in the hope that it will throw light upon this obscure point in cytoryctes.

(*b*) *The zygote.*—Certain definite bodies are frequently found in many different cases which resemble the fertilised female gametocyte of coccidium. These are more or less amœboid forms within the nucleus, characterised by a deeply staining body and a central nucleus-like mass of the protogonoplasm. Occasionally a similar amœboid organism is found in the cytoplasm, one which I observed being surrounded by a very definite capsule and clearly differentiated into cytoplasm and nuclear mass, while the remainder of the cell in which it lay had no trace of parasites. In the nucleus these bodies are spherical, and can always be distinguished from the usual nuclear inclusions by their definite form and dense structure, while the chromatin of the host nucleus is pushed to one side. For the first time the organism now resembles a typical cell, differentiated into nucleus and cytoplasm, for the red-staining central portion of the protogonoplasm now appears as a nucleus, while the cytoplasm is dense and granular and stains uniformly green.

This zygote-like form becomes the pansporoblast and the mother cell of the primary sporoblasts, a process of such moment and requiring such a high potential of vitality that this fact alone justifies the *a priori* conclusion that fertilisation has taken place. There is no visible egg membrane or other morphological structure to aid us in this interpretation. The nuclear body of the parasite at this stage is occasionally double or biscuit-formed, as though dividing.

(*c*) *The Pansporoblast.*—If the nucleus of an ordinary amœba should divide many times, and if a portion of the protoplasm containing a few of these nuclei, while still within the body of the parent amœba, should be differentiated into a sporoblast-forming region, that limited portion would be a pansporoblast. Two, three, or more such limited portions of the cell body might be formed, and each would be a pansporoblast, the parent organism meantime continuing its individual existence. So far as known, this peculiar method of reproduction is met with nowhere but in the sub-class neosporidia of the sporozoa ; and in cytoryctes this stage forms one of the most important phases of the life history, the pansporoblast being the seat of the vigorous "propagative" reproduction.

The adult intranuclear parasite of variola forms but one pansporoblast. At the outset this is a spherical body with very dense cytoplasm, easily distinguished from the nucleoplasm of the host cell, and with a small, spherical mass of protogonoplasm. The protogonoplasm of this stage, which might here be called the nucleus, becomes distributed by fragmentation throughout the substance of the organism. The fragments are so minute at first that they are barely visible as red points within a green matrix

(Borrel stain), and are smaller even than the mature spores. Stages in their growth can be followed, however, and with this growth the contour of the parent organism becomes more and more irregular. The nuclear membrane of the host cell ultimately disintegrates and liberates the parasite, which probably has the power of moving from cell to cell, since nests of ten or twelve are occasionally found. It grows to a comparatively large size (ten microns to twelve microns), and from eight to twenty primary sporoblasts develop within it. In the older organism, vacuoles appear in the cytoplasm and this is the only differentiation.

One large portion of the protoplasm takes no part in the sporoblast formation, but remains undeveloped, and, as "Restkörperchen," gradually degenerates. It becomes irregular in form, and, with the Borrel stain, ultimately changes in colour reaction from red to green. Like the gemmules, the young sporoblasts are solid and homogenous at first, but as they increase in size they become hollow, and in optical section appear as thickened rings. They may be distinguished from the sporoblasts, which come directly from spores, by the uniform thickness of the ring. When these spheres have attained the diameter of about one and a half to two microns, the thickened periphery shows evidence of vacuolisation, which becomes more definite with increase in size.

There appear to be two types of primary sporoblasts, but I am inclined to think that the difference is due to imperfect fixation, or merely an optical effect due to flattening of the usually spherical body. In one, the spores develop in the peripheral vesicles, the central portion remaining hollow; while in the other the spores develop in all of the substance of the sporoblast. A characteristic residual body results from each of these types of sporoblasts, one a mere skeletal framework, the other apparently more solid. In both types the method of spore-formation is the same, the material of the spores, or protogonoplasm, accumulates about the minute vesicles, forming darker spheres lining the spaces, and later it segregates at one pole of each vesicle, forming a more deeply-staining body of the spore, while a characteristic vacuole, composing the bulk of the spore is formed. The peripheral ring of spore-forming substance usually forms vesicles with but one spore each, although vesicles may apparently run together to form larger vacuoles in which three or four spores may lie. Occasionally the sporoblast may be found with the spores immediately outside the broken walls of the vesicles.

The sporoblasts, as in all other forms with pansporoblast-formation, are liberated from the body of the pansporoblast, apparently by the death and disintegration of the parent organism.

(3) *Secondary Nuclear Phases.*—(*a*) *The Spore.*—The following quotation, which I take from Minchin's recent work on the sporozoa, describes the conditions for any germ of the neosporidia. The minute size of the spore of Cytoryctes makes the description particularly pertinent in the present connection, and indicates the possible modes of spore-transportation. "From this point (liberation of the germ) the tiny parasite embarks upon migrations, in some cases very extensive, in order to reach the organ or tissue which is its final destination. It is not possible to state with any certainty how these migrations are either effected or guided. In some cases the journey is perhaps performed on foot, as it were, the little amœboid germ pushing its way actively through the tissues, like a leucocyte. In other cases the parasite may be passively transported by means of the blood current. The latter method is probably the more usual, the little germ being carried along suspended in the blood plasma; at any rate, there is no evidence that it ever attacks the blood corpuscles. The one thing certain with regard to this stage of the life history is that the parasite is able to select and to seek out, in some mysterious fashion, the specific organ or tissue which it affects, and which may be situated at a considerable distance from the original seat of infection."

The spore is extremely minute ($\cdot 57\mu$) and is easily overlooked. Its distinguishing features are the presence of a vacuole, which occupies the greater part of its mass, and a thickened portion at one side. This gives to it a high refrangibility and it shines out with great brilliancy. It stains blue with methylene blue, and red with the Borrel combination, and may be thus distinguished from minute air-bubbles or possible bits of bichloride.

I could make out no trace of the filaments which are characteristic of the majority of neosporidia-spores, but it is not impossible that such structures exist, and that by the use of proper reagents they might be demonstrated. The vesicle, however, is characteristic of microsporidia-spores and it alone is enough to indicate the taxonomic position of cytoryctes.

(*b*) *The Secondary Sporoblast and its Sporulation.*—The intranuclear spore, often embedded in chromatin, developes into what may be called the secondary sporoblast to distinguish it from the primary sporoblast formed in the pansporoblast. This spore at germination might be described as a vesicle with a thickening at one side. As the spore grows the thickened region becomes relatively large, until ultimately it extends entirely around the vesicle. Minute vacuoles then appear within it, first in the original thickened portion, later throughout the entire periphery. Secondary vacuoles may then appear within the first set, that is, towards the centre of the sphere, the entire sporoblast finally becoming a mass of vesicles, some large, others of minute size. These vesicles, both on the periphery and within, are the seat of the formation of spores which are apparently identical with those formed by the primary sporoblasts. The developing spores are at first tiny spheres lining the vesicle, but owing to their minute size, it is impossible to make out their origin. They are formed, apparently, from a substance in the vesicle meshwork, that is, the protogonoplasm which, as in gemmule-formation, again becomes metamorphosed into the reproductive bodies. The vesicles of the sporoblast thus correspond to the chambers of the cytoplasmic form.

In this phase there is, again, no trace of a morphological nucleus, beyond the fact of the more deeply-staining point and the gradual segregation of the protogonoplasm. Division figures were never seen.

With growth of the secondary sporoblast, the nuclear membrane of the host cell becomes more and more faint, until finally it entirely disappears and the parasite is free in the cytoplasm, which also degenerates, leaving the sporoblast in the more or less broken-down substance at the bottom of the pustule.

(*c*) *The Sporoblast Residuum.*—After liberation of the spores from both primary and secondary sporoblasts, there is, as in gemmule-formation, a residual product, but in this case the form is quite definite and the appearance decidedly characteristic. A framework or meshwork would again be descriptive, but there is a far greater homogeneity than in the analogous structure resulting from gemmule-formation, and the general form is retained, apparently for a much longer period. Instead of becoming "stringy," the sporoblast residua eventually swell and become distorted; the periphery finally gives way and the vesicular mass breaks up.

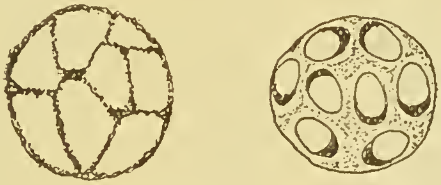

Fig. 1.

An analogous residual structure is described by Stempell (1902) in the sporulation of *Thèlohania mülleri*, but it is passed over without emphasis (see figure 1).

The following diagram (fig. 2) gives a comprehensive view of the life-cycle of the small-pox organism as I have interpreted it. Every stage represented is from a camera drawing of the parasite, the tissue-cells alone being schematised.

C.—Biological Considerations.

(1) *Multiplicative Reproduction.*—Doflein (1898) has given to the endogenous reproduction which takes place in myxosporidia, and by which auto-infection in the host is

brought about, the name "multiplicative reproduction," and to exogenous reproduction, by which the disease is spread to new hosts, the term "propagative reproduction." In a general way multiplicative reproduction is similar to schizogony of other forms of protozoa, but differs from this in certain minor points. Unlike the propagative reproduction, it is always asexual and takes place either by simple binary or multiple division, which Doflein terms "plasmotomy," or else by fragmentation. In the first case the full-grown form with its many nuclei divides into two or more equal or unequal fragments, each of which is multinucleate. In the latter case the nuclear material of a young, immature form breaks up into many fragments by "multiple

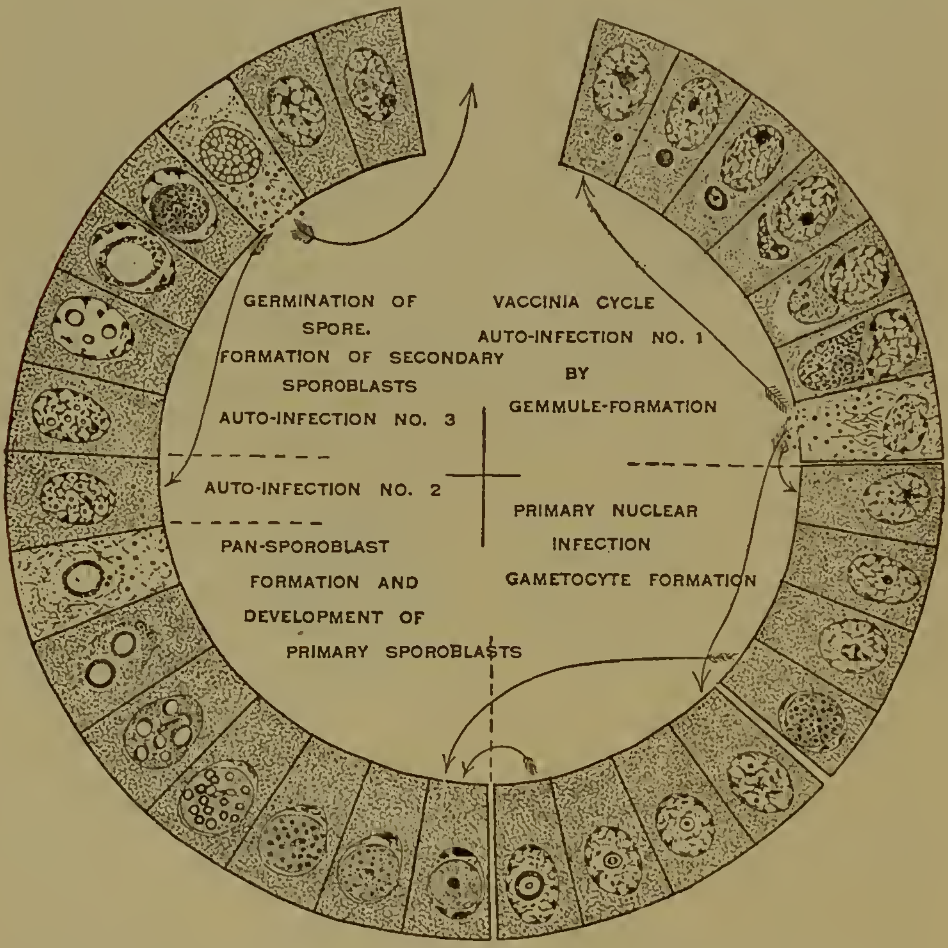

Fig. 2.—The Variola Cycle of Cytoryctes Variolæ, Guar.

amitosis"; the cell-body then breaks up into many uninucleate fragments, which carry the disease into new regions of the tissue or to new organs.

This method of reproduction is accepted as very common, if not universal, among the tissue and cell-infecting forms of neosporidia. In *Thèlohania müllerí* (L. Pfeiffer), according to Stempell (1902), a more involved method of multiplicative reproduction occurs, which Minchin considers intermediate between multiplicative increase by simple division, and by "multiple amitosis." The young forms of the parasite divide after direct division of the nucleus, but the daughter individuals remain connected and divide again without separation. Thus, a chain of individuals is formed which breaks up only later.

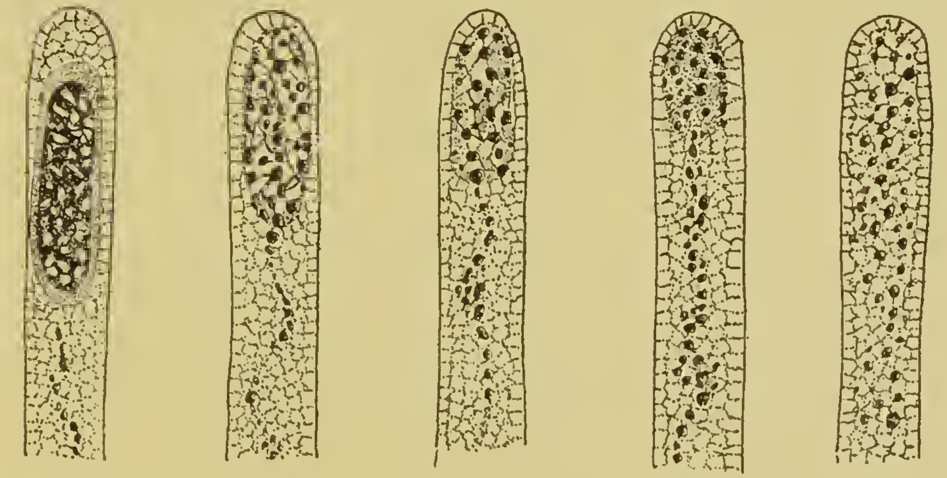

FIG. 3.

Students of the protozoa will recognise the fact that in the process of gemmule-formation I have described a method of reproduction which, so far as known, is not duplicated in any other protozoon. Auto-infection in sporozoa is usually brought about by a process of sporulation, and the spores thus formed, technically known as merozoites, are similar in form and size to the sporozoites, the essential difference between them being the mode of origin, which is asexual in the former, sexual in the latter. In the well-known forms there is thus an alternation of sexual and asexual generations—several of the latter (as in malaria) to one of the former. The process of gemmule-formation, however, belongs to the same category of reproductive phenomena as the multiplicative reproduction of myxosporidia and of other microsporidia. Thus Doflein (1898) observed the myxospore of *Chloromyxum leidii* forming daughter individuals by division, and recognised a similar mode of increase in *Hoferellus cyprini*, while Laveran and Mesnil (1802) observed the multiplication into many parts of *Myxidium lieberkuhni*. It was early noticed by Cohn that this process occurred mainly during the winter months, and in this period the customary spore-formation was in abeyance. Although the students of the microsporidia (Thèlohan, 1895, Doflein, 1898, Lühe, 1900) recognised the necessity of such a process to explain the enormous increase of the parasite while within the host, with the exception of Thèlohania there have been no positive observations on this type of multiplication in forms allied to Cytoryctes. It is obvious, however, that some mode of reproduction, apart from schizogony and sporogony, might be expected in the small-pox organism.

The experiments of Guarnieri and of Wasielewski show conclusively that reproduction of the cytoplasmic phase must take place, and our observations on the structures of these forms in the late adult stages fully confirm the *a priori* conclusion. The method, however, appears to be almost unique in animal forms, although similar processes occur in bacteria. There is some evidence to justify the view that multiplicative reproduction in Cytoryctes may occur through simple division of the larger amœboid stages, and forms are frequently found of dumb-bell shape, or with thin strands of protoplasm connecting two larger masses. It is possible that the multiple infection, sometimes seen in a single cell, may be due to this method of increase, but I do not think that this is an efficient means of reproduction. Gemmule-formation is a much more prolific method, and satisfies the *a priori* requirements based upon the assured facts of distribution in the host and upon the potency of vaccine virus, in which the cytoplasmic phase is the only organism found (Councilman). The gemmules, therefore, are of the nature of bacterial spores, and, like the latter, are probably capable of withstanding unfavourable external conditions. The method of their formation, furthermore, is analogous to the formation of spores in bacteria. Here, according to Bütschli, Schaudinn, and other observers, there is no nucleus, but the cell contains scattered granules (protogonoplasm ?) of what appears to be nuclear material, identical, apparently, with the distributed granules in Cytoryctes. Schaudinn (1902) has clearly shown how this granular material collects at one end of *Bacillus bütschlii* to form the spore, and he calls attention to the fact that, when thus accumulated, the mass resembles a morphologically differentiated nucleus (see figure 3, p. 163). The apparent nucleus, however, develops into a complete bacillus. So in the case of gemmule-formation in *Cytoryctes variolæ*, where the protogonoplasm collects in minute spherical masses, which, like the spore of *Bacillus bütschlii*, may develop into organisms like the parent, but, unlike the spore of the bacillus, they may in time develop into sexual organisms which are dissimilar to the immediate parent and have a definite reproductive function. Unlike *Bacillus bütschlii*, again, Cytoryctes forms not one but many of these reproductive bodies, and in this respect simulates the polysporous bacteria. In asexual reproduction, therefore, it may be stated that Cytoryctes approaches more closely to the bacteria than to the other forms of parasitic protozoa.

The amazingly wide-spread infection in variola, an infection which, within a comparatively short time after exposure to the disease, may involve nearly the entire surface of the human body, can be explained only by the assumption of both rapid and varied modes of increase of the parasite. Auto-infection by gemmule-formation is one such mode of reproduction. Another, and an even more potent method, is by schizogony or direct development of the spores into secondary sporoblasts. In some small-pox cases there is a notable absence of adult organisms in the epithelial cells. In many nuclei of these cells, however, there is at least one, while there may be three or four spores. In the protocols such cases are described as aberrant, and the virulence may be due to this wide-spread infection. Here the definitive environment is not the cytoplasm, nor the general nucleoplasm, but the specific chromatin substance within the nucleus. It appears, then, that we have in this phenomenon another interesting biological fact, viz., " chemiotactic selection " of chromatin, analogous to " chemiotactic selection " of blood corpuscles by the malaria organisms, or selection of nucleoplasm by *Cyclospora caryolytica Schaudinn*, or similar selection by numbers of sporozoa of certain definite tissues of definite hosts.

How the spores get into the nucleus is a matter of speculation. We know that the effect is produced, and we find the definite spores in this position. The spores (0·57 μ) are so minute that they approach well within the possibility of Roux's " invisible germs," and it requires no more stretch of the imagination to account for their wide distribution than it does to account for an equal distribution of some toxin produced by an organism. We have described above the germination of such spores and the direct development of the secondary sporoblast, a most unusual phenomenon, whereby the primary sporoblasts, which are formed in the pansporoblast, are duplicated by direct development of spores. So far as I know, the only similacrum is the reproduction of trematode worms where larval rediæ give rise to similar rediæ. This process, together with that of gemmule-formation, is sufficient to account for the wide-spread infection which characterises variola.

(2) *The Distributed Nucleus.*—It is generally known that many unicellular organisms, *e.g.*, the bacteria, have no definite nucleus. This does not mean, however, that nuclear structures are absent or that Hæckel's group of enucleate organisms (monera) is justifiable. A morphological nucleus, which to many recent critics (*e.g.*, Foa, Doflein, &c.), appears to be the *sine quâ non* of an intracellular parasite, is not only not necessary, but not to be expected in these lowest forms of sporozoan organisms. Critics who deny the appellation " organism " to certain cell inclusions, on the ground that there is no nuclear differentiation, are either ignorant of

the facts or wilfully overlook them. As a matter of fact, it is generally conceded by cytologists at the present time that nuclear material, presumably chromatin, may be present in the cell in other forms than that of a morphologically differentiated nucleus. This occurs more and more frequently as we approach the lowest forms of living things, until, in the cyanophyceæ and bacteria, it is the prevailing condition. Schewiakoff (1893) was among the first to show that such a condition is normal, and in the case of Chromatium he demonstrated that the distributed granules can reproduce themselves by division. In the bacteria the granules are distributed throughout the cell body, a fact which led Bütschli (1890), at first, to regard the entire cell as a nucleus. In division of bacteria these granules are passively separated into two groups, but in spore formation they become aggregated to form the body of the spore (see Schaudinn, 1902, and above, p. 165).

An intermediate condition occurs in some flagellates ; in Tetramitus, for example, the distributed granules collect prior to cell division and are divided into two equal portions while thus aggregated, while in Chilomonas they are permanently aggregated, although without a membrane. From this condition intermediate steps to the formation of definite morphological nuclei have been traced in the different types of protozoa.[1]

In the malaria organisms the nucleus cannot be regarded as a morphologically differentiated structure, for it frequently appears as a mass or masses of chromatin material (see fig. 4, p. 168). Finally, in Lymphosporidium truttæ, which caused a most destructive epidemic among the brook trout in a hatchery on Long island, a similar distributed nucleus occurs.

The term " distributed nucleus " was proposed by myself (1898), and interpreted as a primitive condition of nuclear substance before a definite morphological nucleus is differentiated. Hertwig (1902) proposed the term " Chromidien " for such distributed chromatin, and he, followed by

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[1] See in this connection Calkins, 1898, 1901, 1902.

Schaudinn, also regarded it as a primitive condition of the nuclear substance. As the term "chromidium" is already in use by the botanists to designate a very different structure, I have replaced it in this paper by the term "protogonoplasm."

(3) Systematic position of *Cytoryctes variolæ* and description of *Caryoryctes cytoryctoides*, nov. gen. et spec.—So far as I am aware, there is but one other organism

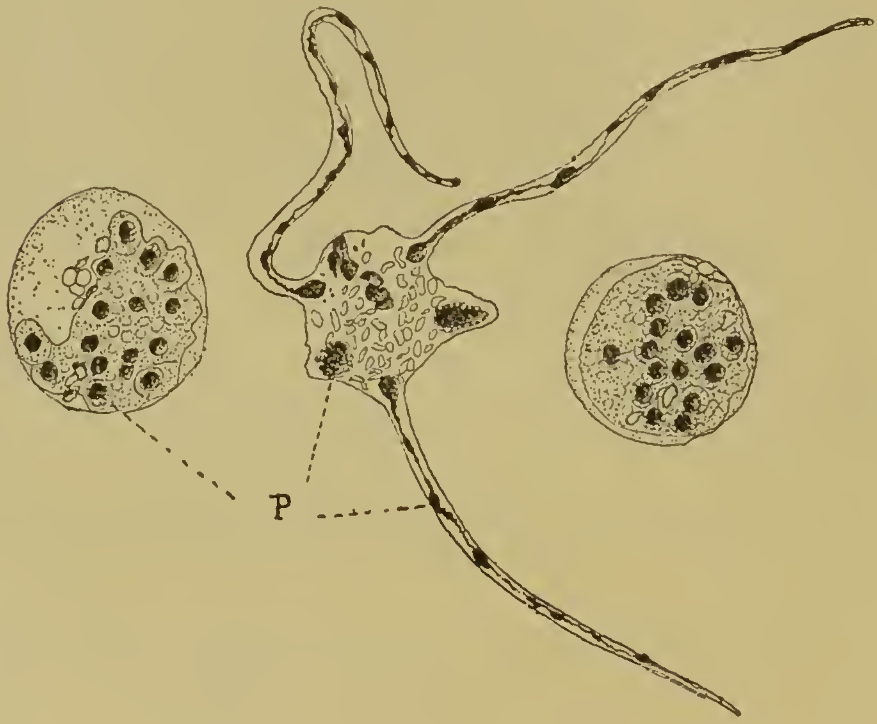

FIG. 4.

which is similar in structure to the intranuclear parasite of small-pox. This is an intranuclear parasite of the macronucleus of the ciliated protozoon, paramœcium caudatum. In 1896 I made preparations from a mixed culture containing many of these infusoria, and noted at the time curious inclusions within the macronucleus. Little attention was given to them, and the preparations were laid aside. After seeing the intranuclear forms of cytoryctes I re-examined these preparations, and was struck by the close similarity between the two intranuclear forms. In none of the cultures of paramœcium examined before or since have I found a like condition. The organisms of these preparations are beautifully fixed, every part is normal, and, except for the spaces in them in which the parasites lie, the macronuclei are characteristically homogeneous. The inclusions are no degeneration products, nor are they products of secretion. They are organisms, and protozoa of a definite kind, undoubtedly belonging to the same group as *Cytoryctes variolæ.* On account of the possible importance in comparison with the small-pox organism I shall name this genus caryoryctes, and from its close resemblance to the latter, I shall give it the specific name " cytoryctoides."

The development of the spores of caryoryctes may be traced in different specimens of paramœcium, and shows a close agreement with spore development in cytoryctes. The sphere enlarges, the collection of plasm at one side of the spore becomes vacuolated, and new spores are ultimately formed. Like spores in cytoryctes, these lie in minute vacuoles on the periphery of the sporoblast.

As in the latter, again there are great residual structures with clear vacuoles, and skeleton frameworks which suggest Stempell's figure of the residuum of Thèlohania (fig. 1, p. 161). In one case a condition is apparent which may be analogous to gemmule-formation in cytoryctes; a few of the vacuoles contain solid spherules, while the majority are empty. The scarcity of material makes it impossible to draw positive conclusions as to the complete life-cycle, and to establish the complete harmony with the cycle of cytoryctes.[1] There are enough analogous stages, however, to convince us that we have to do with an organism similar to that causing small-pox, and the nature of the single-cell host dispels any shadow of doubt that these structures are artefacts or degeneration products.

The known species of microsporidia are provided with more or less definite nuclei. This feature, therefore, separates the family nosematidæ (with which our organism seems to be most closely allied) from *Cytoryctes caryoryctes* and, probably, lymphosporidium. With the exception of the last, the spores have the characteristic vacuole of the microsporidia (see figure 5), and I see no reason why these organisms should not be included in the tribe polysporogenea (Doflein). Under the family name of cytoryctidæ, I would place them next to the nosematidæ in Doflein's classification. Should other organisms be added to these, it is not unlikely that the characteristics will be sufficiently definite to warrant an ordinal grade, or, possibly, a still higher grade in classification. Such a group, whatever it is, which includes these forms would be the lowest of the sporozoa, and would occupy a position between the parasitic bacteria on the one hand, and the higher sporozoa on the other. For the present I do not think it advisable to go as far as this, and will place them provisionally as follows :—

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[1] The two preparations which I have from this particular culture contain about 375 individuals, of which about 80 per cent. are infected.

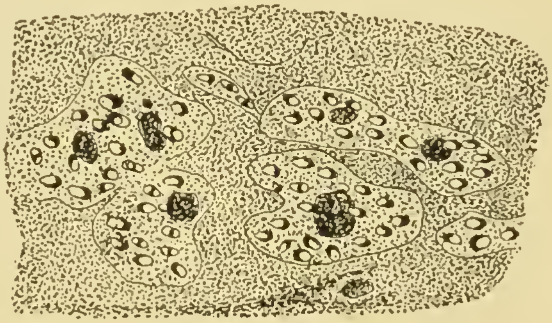

FIG. 5.

Class Sporozoa.

Sub-class myxosporidia (for sub-divisions see Doflein).

Sub-class neosporidia.

Order microsporidia.

Tribe polysporogenea :—

(*a*) Family nosematidæ. (The body continues to grow during sporulation. Many pansporoblasts, each without a membrane. Nuclei present.)

(*b*) Family plistophoridæ. (Body completely used in the formation of the pansporoblast, with membrane. Nuclei present.)

(*c*) Family cytoryctidæ. The organism forms one pansporoblast which is without a membrane. Nuclei absent.

(1) Genus Cytoryctes, the cause of variola.

(2) Genus Caryoryctes, the paramœcium parasite.

(3) Genus Lymphosporidium. Brook trout parasite.

In conclusion we may summarise our knowledge of cytoryctes as follows :—

We know :—

(1) The gemmule and its growth, leading to :—

(2) The cytoplasmic amœboid adult.

(3) The process of gemmule-formation and auto-infection, No. 1.

(4) The residual cytoplasmic structures.

(5) The development of the pansporoblast.

(6) The development of the primary sporoblast and auto-infection, No. 2.

(7) The spore and its germination.

(8) The development of the secondary sporoblast and auto-infection, No. 3.

(9) The method of spore-formation.

(10) Structures which appear like fertilised eggs-cells.

(11) Structures which appear like developing microgametes.

(12) The residual structures of nuclear origin.

(13) Similar organisms parasitic in nuclei of other animals.

We have evidence, but need more light upon :

(1) The actual entrance of gemmules into the nucleus.

(2) The significance of the intranuclear sporoblast following upon the adult cytoplasmic amœboid form.

(3) The origin of the pansporoblast mother-organism. These may possibly all be summed up in :

(4) The sexual phenomena.

We are ignorant in regard to :

(1) The first stages of the organism in a new host.

(2) The transportation of the infecting agents by the blood.

(3) The method of cell and nuclear infection.

(4) The significance of the inhibition of the nuclear phases in the vaccine-organism.

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EPICRISIS

BY

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In the early stage of the specific lesions of the skin and mucous membranes in small-pox, bodies are found which vary in form, structure and size. We regard these bodies as the parasites causing the disease. They occur within the epithelial cells, within the nuclei and free. The forms within the nuclei are subsequent to those which develop within the cytoplasm. They are present in the greatest numbers in cases of the greatest severity and rapidity of course. They do not occur as isolated structures, but one form follows another by gradual transitions, forming a cycle which corresponds with the cycle of development of living things. In the different cases the same forms are found at the same period of the disease. The bodies increase rapidly in the lesions and the lesion increases in extent by continuous infection of adjoining epithelial cells. The same forms are found in corresponding situations in the lesions in different cases.

Bodies simulating those which occur in small-pox may be found in other diseases. In all of these the bodies represent an accidental product. There is no complexity of structure such as is found in most of the small-pox bodies. There is no sequence representing growth development. These accidental cell inclusions simulate most closely the early forms of the cytoplasmic bodies before structural differentiation is apparent. Such inclusions are the product of a number of conditions.

Red blood corpuscles may be carried into the epithelium of mucous membranes and are found both between and within the cells. We have found this, however, only in cases where there was abundant hæmorrhage and œdema in the underlying tissue, and where the cells were separated, allowing the exudate to freely pass through. The most striking example of this was found in the soft palate in a case of diphtheria. The red blood corpuscles retain their characteristics, though single corpuscles may break up into two or more pieces and should not be confounded with the parasites. There is more chance of confounding leucocytes or their products with the parasites, but any one with the usual knowledge of forms of degenerating leucocytes should be able to recognise them. These sources of error can also be dismissed, because red corpuscles are not carried into the intact epidermis by exudation streams, and leucocytes are absent in the earliest lesions in the skin where the cytoplasmic forms are found in the greatest numbers. The possibility of confounding certain artefacts which are formed in the red blood corpuscles with intranuclear forms of the parasites should be considered. By hardening red blood corpuscles in Zenker's fluid or sublimate, staining and imperfectly dehydrating them, an appearance of small refractive vacuoles, many of which contain a minute dot, is produced in them. We have rarely found red blood corpuscles in a section presenting this appearance, but it is easily produced in a coverslip preparation of the blood. There might be a possibility of confounding certain forms of the parasites with the nuclear detritus from lymphoid cells, but these cells are rarely found in the epidermis, and nuclear detritus from them occurs only in the pustule contents.

Various conditions simulating the parasites may also be seen in vaccinia. In describing the vaccine bodies in the cornea, Dr. Tyzzer calls attention to the swellings on the terminal nerves which might be confounded with the parasites. Occasionally in the epithelial cells of the cornea a space is formed around the nucleus by shrinkage, and this space may contain a coagulum. The protoplasm of the epithelial cells may stain more sharply immediately around the nucleus, and in various ways suggestions of the specific inclusions may be given. We have never seen such formations in the skin lesions of small-pox.

The morphological products of cytoplasmic degeneration do not simulate the parasites. Such products are usually multiple, devoid of internal structure and have no fixed relations with the cytoplasm, such as a vacuole around them. Chromatin particles arising from nuclear fragmentation and enclosed in the cytoplasm cannot enter into consideration, for the nuclei of epidermis cells do not fragment in the same way as the more solid nuclei of the mesenchymatous tissues. Moreover, the cells in which the youngest forms of parasites are found have morphologically normal nuclei and cytoplasm. We have in another place called attention to the fact that the earliest degeneration is found in the more superficial cells of the epidermis, while the parasites are in the lower. Specific products of epithelial cells, such as kerato-hyaline granules, are too numerous, have no definite size, and are formed in parts of the epidermis where parasites are rarely found. The parasites in both variola and vaccinia have been attributed to degeneration of the centrosome. No proof has even been given that the centrosome does degenerate. If the centrosome could by any process of degeneration give rise to such a product as any one of the cytoplasmic parasites, it would have to increase enormously in size and change all its characteristics. The parasites are also found between cells and within cells in mitosis, in which latter it may be assumed that the centrosome is normal.

The young cytoplasmic parasites may be closely simulated by certain products of nuclear degeneration. We have spoken of the clumping of chromatin in the nucleus as a common form of degeneration in various epithelial organs. It takes place in some situations more readily than in others. The mass collects in the centre of the nucleus and may be round or irregular in form. In the eosin and methylene blue stain it frequently changes its staining reaction, becoming more refractive and taking a pale lilac, which gives place to a bright eosin stain. Such masses may pass from the nucleus without evident injury of the nuclear membrane. In the cytoplasm they have the size, the refraction, and the staining reaction of the young undifferentiated cytoplasmic parasites. We have never been able to make out the passing of the chromatin from the nucleus in any of the small-pox lesions, though it probably does do so. In diphtheria, in the degenerated epithelial cells beneath and at the edge of the diphtheritic membrane this process undoubtedly does take place. We have specimens in which this is shown as clearly as is the migration of leucocytes. The chromatin when it passes into the cytoplasm lies near the nucleus and not in a vacuole.

The larger of the cytoplasmic parasites are more characteristic than the smaller forms, though it is possible to confound these with products of degeneration. In advanced degeneration of the epithelial cells found at the edge and at the bottom of vesicles, masses of separate cytoplasm, often intermingled with shreds of fibrin, may be found which offer a remote similarity to the large forms. There is no doubt that single objects formed in various ways may be found in the cell cytoplasm in small-pox and in other diseases, which may simulate to a greater or less extent any of the forms of the cytoplasmic parasites. We have often found such single objects in small-pox and been unable to say whether they were or were not parasites. We think it possible that some of these represent abnormal or degeneration forms of the parasite. Every one who has had any experience with the study of unicellular organisms in tissues knows that it is often impossible to say whether or not a single object belongs to the host cells or is a parasite. Not only must form and size and structure of the single individuals be studied, but their relation to their surroundings and their developmental cycle must always be borne in mind.

Many points in structure which have been described are only to be distinguished after long study and in the best preparations. Careful staining by the methods which have been described is essential and requires considerable practice. We have found photography of enormous assistance, and have often become aware of certain details of structure only after the study of negatives. Details can also be made out better by the aid of a properly screened arc light than by any other form of illumination. The differentiated structure in the cytoplasmic forms of from 2 to 3 microns in diameter was first seen in the negatives. There is no similarity in size, structure and relation to the cell between the cytoplasmic parasites and the various forms of cell inclusions in tumours, and the study of small-pox has thrown no light on the question whether the inclusions in tumour cells are or are not parasites.

The intranuclear parasites are less apt to be confused with accidental products than are the cytoplasmic. They are found in nuclei in which there is no change in the chromatin, and they stand in no relation to the chromatin. Certain degenerations of chromatin may present some similarity to certain forms of the parasite. In the clumps of chromatin formed in the centre of the nucleus small, clear, highly refractive vesicles may be found which suggest the chambers in the sporoblast. Pianese, in his valuable studies of the histology of carcinoma, has described and depicted various conditions within the nucleus which somewhat resemble certain of the intranuclear parasites. We have never found forms similar to those depicted by Pianese in the epithelial nuclei in variola or in any other infection of the skin. The vacuoles in the chromatin clumps are much more apt to be found in the mucous membranes, particularly in the œsophagus, than in the skin. The best example of this formation was seen in the nuclei of degenerated muscle fibres in a case of fibrous myocarditis. It would be easy to say that the sporoblast could represent a more extensive formation of the same sort, that the vacuoles enlarge and form the chambers, and the remains of the chromatin represent the skeletal framework. A process very similar to this does take place in the testicle. We have already spoken of the peculiar bodies which are found in the testicle and which arise in connection with aspermatogenesis and from degeneration of the chromatin of the spermatids. Many of these bodies closely resemble the younger forms of sporoblasts, but they are formed from chromatin and stain like this. They represent merely a skeletal framework of chromatin enclosing vesicles. They are formed of the nucleus, while the intranuclear parasites grow within the nucleus and have no connection with its constituent parts. No structures resembling the completed sporoblast are so formed. Each form of the intranuclear parasite must be studied, not as an isolated object but as a stage of a cycle and by comparison with the forms which precede and follow it.

In vaccinia we find the same forms of parasites as in the cytoplasmic cycle in variola. None of the intranuclear forms have ever been found in vaccinia in any animal used for inoculation. We have never had an opportunity to examine the developing vaccine vesicle in man, but we have no reason to suppose that the lesion in man would differ from that in the monkey. In vaccinia there is some difference in the size and in the rapidity of development of the parasites in the different tissues and in the different animals. Their development seems to be most typical in the skin and mucous membrane of the calf. In the cornea their development is less perfect. They correspond in size, structure, and manner of multiplication with the cytoplasmic forms of the variola parasites in man and in the monkey. There are minor differences easily accounted for by differences in the soil in which they grow and their better preservation in the tissues of animals. The differences are not greater than will be shown by the same flowers in different gardens.

We believe that these bodies in vaccinia and in variola are living things. We see no possibility of another conclusion. Otherwise we must assume that they are degenerations of a specific character occurring under no other conditions, and that the products of degeneration undergo a development similar to that of a living thing, increasing in size and complexity of structure, and finally breaking up into a number of forms similar to those met with in the beginning, and which undergo the same development. Moreover, the manner of development constantly repeats itself, and similar stages are found at similar time periods. We know of no such degenerations, and all that we know of pathological processes is against such an interpretation. For the view that they are living things we have the analogy of structure and development with other things which are generally conceded to be living. The supposed parasites of variola and vaccinia appear first as bodies of small size, but not as microscopic points. They increase in size, and, with growth, details of structure appear which are always repeated, and for which the time relation, as far as can be determined, is the same. At the end of growth a form of multiplication takes place. The absolute proof that an object is living is very difficult to produce, for there is no single criterion which suffices. Amœboid motion has been made out in the vaccine parasites by so competent an observer as Wasielewski, but although believing that the bodies are parasites, he very properly does not regard this as proof.

Assuming these structures to be parasites, are they to be regarded as the cause of the disease? For this, also, it is impossible to produce actual proof. It is impossible to prove that the bacteria which are found associated with lesions of certain disease are their cause. Certainly every one believes that the pneumococcus has a direct causal relation with lobar pneumonia, but the proof is absent. The same is true of the leprosy bacillus and of the typhoid bacillus. The view that the *Cytoryctes variolæ* is the cause of variola must for the present rest on the fact that it is always associated with the lesions of the disease, developes further as the lesions develop, and is found under no other conditions. Every analogy which can be drawn from the study of other infectious diseases, both those in which it is possible to fulfil Koch's law, and those in which this is not possible, supports the view of its casual relation. It is not too much to say that an organism which has the power of invading and destroying living tissue, and which is constantly found in relation to the developing lesions of a certain disease, and only here, and unaccompanied by other living organisms, must be regarded as the cause of the disease. We have constantly found the organism in connection with the developing lesions of the disease.

After the lesions have reached their full development, after local immunity is established, and the host cells are no longer capable of affording a suitable place for development, we do not find the organism. It is an obligate parasite, it developes within the living cells of a tissue, and is only found in connection with these. Those which are found free in the tissue are in the bottom of the vesicle or pustule, and have become free by the complete destruction of the cell in which they developed. These free forms represent the skeleton framework of sporoblasts in which spores have developed. In the contents of vesicles and pustules examined by smears and by sections, and by every technical method we could apply, we have not certainly found the organisms, though we know that the material examined is infectious. It has been equally impossible to say that the organisms were absent. To show them present we must be able to differentiate bodies of extremely minute size, from 0·37 μ to one micron, which have no characteristic staining reaction, from the mass of small objects, such as bacteria, cell and nuclear detritus, and granular precipitates from the exudate, which the pustule contains.

Bacteria are also usually found in the lesions. All the varieties found have been cultivated and the impossibility of their causal relation shown. Their presence is not constant. Wassermann first showed by cultures that pustules could be free from bacteria, and we have been able to confirm this in several instances. The histological study of the pustules shows bacteria to be not constantly present and usually in very small numbers. They give the impression, from their location in the lesions, of being only accidental. Perkins has found the tissues free from bacteria in a case of purpura variolosa in a child. We have further shown that variola can be produced by material in which bacteria were demonstrated to be absent by carefully carried out cultural methods.

We believe that variola and vaccinia are due to the same organism. It has been repeatedly demonstrated that vaccinia can be produced by inoculating cows with variola.

As has been said, in vaccinia only the cytoplasmic forms of the parasite are met with, and these occur in the calf and rabbit with the same regularity, whether material from vaccinia or small-pox has been used. Vaccinia, with only the cytoplasmic form of parasite, is produced in the monkey when vaccinia is inoculated. When the monkey is inoculated with small-pox both the cytoplasmic and intranuclear forms of parasites appear. In man we know that both forms appear in small-pox, and we have not had opportunity for the study of vaccinia in man. We believe that the cytoplasmic and intranuclear forms of the parasites represent each a distinct cycle. The cytoplasmic cycle is perfectly simple, and no forms are found in it which can be interpreted as sexual. The intranuclear cycle, found only in variola, follows the cytoplasmic, is infinitely more complicated, leads to a more active multiplication of the organism, and to the production of forms which can be air-borne and are capable of producing infection without intermediate agents. That this second cycle is sexual in character is extremely probable, though we have not positively identified the sexual forms. We have made no experiments as to the comparative resistance and durability of the vaccine and variola virus, nor do we know that any have been made. From analogy with other organisms it would seem probable that the products of the asexual cycle in vaccinia should be less resistant and in the absence of sexual rejuvenation should gradually decline in infecting power. There are some vague statements in literature that the efficiency of a strain of vaccine does decline in the course of years, but there is no positive information to this effect. We have repeatedly failed to get positive results on the cornea from inoculations with various sorts of commercial vaccine, but have never failed to get typical positive results with vesicle and pustule contents of small-pox lesions. The future experimental study of the diseases on an animal susceptible to both can alone decide these points.

Of the mode of infection in variola we are ignorant. The hypothesis which seems most plausible and which is most generally held is that infection takes place by the reception of the air-borne virus on the respiratory mucous membrane. The organism increases in the favourable soil and produces a local lesion, the so-called protopustule, from which the infection of the blood takes place. Blood infection is marked by a sharp onset, and the skin eruption is embolic in character. No lesion in the respiratory mucous membrane which could be regarded as an infection-atrium, a protopustule, has even been seen. In all of our cases a careful search for such a lesion was made. There are many grounds against the assumption of such a protopustule. The period of incubation which should correspond with development of the protopustule is, in the great majority of cases, entirely without symptoms. We know, however, that very considerable lesions in the lungs may not be accompanied by any symptoms. We see this particularly in tuberculosis. It is not impossible that the lesion may have gone through its development and healed without leaving a recognisable trace. It is extremely improbable that the protopustule could be formed in the lungs, for no specific lesions of the disease are found in them. There is every opportunity for their infection both from the surface and by the blood during the disease, and we cannot think that immunity limited to an organ was produced by the primary infection. In many cases we find losses of substance in the pharynx and other mucous membranes which might be the remains of an initial lesion, but they may be absent. Since the eruptive lesions in the mucous membranes have the same general character as those of the skin, analogy would lead us to believe that a primary lesion in a mucous membrane would correspond to the lesion of the skin which follows a primary inoculation. Judging from the character of the initial lesion produced on the monkey by inoculation, it would seem impossible that a primary lesion in the pharynx or on any other mucous membrane could run its course without symptoms. Cases have been known in which accidental infection of the skin and mucous membranes followed by the eruption have taken place. So far as we have been able to judge these cases from their reports, they have taken the course of variola inoculata and not that of true variola. An interesting case has been brought to our notice, in which a primary lesion of the hand, followed by a typical eruption, occurred after shaking hands with an individual who had taken care of a small-pox patient. In variola inoculata in man a primary lesion develops which is followed by the eruption. We see the same in the monkey. But variola inoculata has a different history from variola vera. The period of incubation is shorter and the disease incomparably milder. Variola inoculata in man has not been studied since the introduction of the thermometer and careful clinical methods. In man and in the monkey local reaction following inoculation is manifest on the fourth or fifth day. The constitutional reaction in man appears on the seventh or eighth day, but is shown in the monkey by rise of temperature on the third day and before the development of the local reaction. The exanthem appears in the monkey on the eighth day, and in man on the eleventh. The contents of early vesicles were used for inoculation in the production of variola inoculata, and the possibility cannot be excluded that undeveloped organisms were used. Our experiments have not been sufficiently extensive to enable us to say that there is a difference in the course of the disease depending upon the stage of the lesion from which the virus is taken. It must be insisted upon that variola inoculata is not variola vera, and that we know nothing about the mode of infection in the latter.

From the contents of a small-pox vesicle or pustule three forms of the same disease can be produced ; (1) in the calf and in the rabbit vaccinia. This is to be regarded as a true variety of the disease, which, when established, always comes true from the seed. There seems to be some evidence that the variety is not established at once, but that the parent stock can be produced in suitable soil after the first generation. The virus is not air-borne. (2) Inoculation of man and monkey with the same material produces variola inoculata, a form of disease not so sharply marked as vaccinia, which cannot be established as a variety, and which is distinguished from variola vera by its shorter period of incubation and its milder course. It is only by rare accident that opportunity will be again given for the study of the disease in man ; we know the disease only from the meagre records of the past. (3) Infection of man by natural and unknown means produces variola vera, the type of which is well characterised. There is no evidence that variola vera is ever produced in the monkey. The details of Zueler's case, in which he infected a monkey by placing in his cage small-pox material, are not sufficiently definite to enable us to say that the disease was not variola inoculata. Any one form of the disease produces immunity from all other forms.

It is generally assumed that the eruption is embolic in character and follows the blood infection. It is difficult to explain it in any other way, but it is equally difficult to understand the progress of the eruption by this assumption. The eruption appears first on the face and next on those parts of the skin habitually uncovered. An exception to this rule is the skin over the instep, where the eruption is abundant and early. It is possible that the skin infection is simultaneous, and that certain parts, owing to increased vascularity or some unknown condition, allow a more abundant and earlier development. The vascularity of a part certainly seems to have an influence. Parts of the skin surface which have been rendered more vascular by the application of a mustard plaster or any slight trauma almost certainly have a more abundant eruption. The eruption may be greatly influenced in other ways. A young man of twenty in the period of incubation and one day before the onset was operated on in the City Hospital for bilateral hammer toe, and both feet and ankles put in a corrosive dressing. The eruption was abundant and took the usual course. The dressing was not removed until the fourth day of the eruption, when the skin beneath it was found normal. Parts subjected to friction of clothing or where occupation subjects the skin to friction, as the shoulder of a violin player, have a more abundant eruption. It does not seem probable that this can be merely a matter of increased vascularity which gives a greater opportunity for infection, possibly combined with more favourable conditions for the organism. Parts which are habitually subjected to friction, as the axillæ, are comparatively free from the eruption. It would be extremely interesting in this connection to know the distribution of the eruption in people habitually naked, but on this we have found no observations. In other eruptive diseases it is equally impossible to understand the distribution of the eruption. There is little evidence of the infectiousness of the blood derived from inoculations. The only time when this would seem most probable is at the end of the period of incubation and at the beginning of the onset. In two light cases inoculations of the blood on the rabbit's cornea were made during the period of onset and before the eruption, with negative results. In these cases it is possible that the parasites may have been contained in the blood in such small numbers that the chances would have been against a positive result. The only positive result of corneal inoculation with blood was in a case of purpura variolosa in which the parasites were so abundant in the skin that the possibility of contamination of the blood must be considered. Support for the theory of blood infection at the period of onset is also given by fœtal small-pox. In this the lesions of the fœtu- are described as twelve days later than those of the mother, the fœtal infection being supposed to take place at the time of onset in the mother. The lesions in the case of fœtal small-pox which was among our autopsies and those seen in Dr. Engleman's specimens were considered due, not to the parasites, but to toxic substances passing from the mother to the fœtus. The lesions were atypical and no organisms were found in them. Since then another case of fœtal small-pox has been studied in which the lesions were typical, and in them all forms of parasites were found. The infection of the fœtus must take place by means of the mother's blood, but in our case it was not possible to determine the period of fœtal infection. Support is also given to the embolic theory of the skin eruption by the observation of the cornea. We have never seen at autopsy specific lesions on a cornea, nor have we found certain descriptions of specific lesions in the literature. Dr. Bancroft has, however, seen a typical lesion develop on a cornea which had become vascular from old disease.

Although bacteria can be excluded as a primary cause of small-pox, they play a very prominent part in the pathology of the disease. As the results of our study of the disease, both by cultures of lesions and organs, and by the microscopic examination of tissues, we are inclined to regard bacterial infection as a more important agent in bringing about a fatal termination than the specific parasite. It is impossible to say to what extent the lesions in internal organs are due to bacteria and their products. Some of them, notably the changes in the blood and blood-forming organs, and those in the testicle, we are inclined to regard as specific and due to the action either of the specific parasite or toxins produced by it. We have never been able to demonstrate the parasites in the testicle, but this is by no means proof that they are not present. The degenerative lesions are very much the same as in other infectious diseases in which there is a combined bacterial infection. There are some differences, but apart from the condition of of the blood-vessels we do not think it would be possible to make a certain diagnosis of small-pox from the examination of the liver and kidney. There is also evidence of the absence of tissue inhibition to bacterial invasion and growth given in the abundance and character of the growth in the tissues, apart from the observations of a diminished complement. The bacteria are chiefly streptococci. There is analogy in respect to the importance of the part played by bacterial infection in the other exanthemata, notably in scarlet fever.[1]

It would be idle to discuss further the general pathology of the disease. In various articles in the series the general application of the knowledge obtained from investigation is given to some extent.

We hope that our work will lead to a greater interest in the disease, more investigation, and more knowledge. Small-pox is the most perfect type of an infectious disease which we have. It has features which are unknown in other diseases. It is not impossible that in the other exanthemata immunity against the true disease will be found in the production of a mild variety which repeats itself. All that we can learn of small-pox may be of immense importance in combatting other exanthemata. Opportunities for its study will be present in places where they can be taken full advantage of. There has been but little study of the disease since 1873 until the present century. The most important, if not all, forms of the disease can be studied experimentally, and experimental study must go hand in hand with study of the disease in man. The recognition of the specific cell and nuclear inclusions as parasites, and the primal cause of the disease, must come to any one from careful study. It would be too much to expect that a cycle of development of the parasite complete in all its details should be given at once. We can see no objections to the cycle given by Professor Calkins, but there is much uncertainty about the interpretation of many of the forms, particularly of the sexual forms. The most important and immediate practical result of the work should be the testing of the quality of vaccine virus by rabbit inoculation. As a means of diagnosis in obscure cases of variola, rabbit inoculation may be of great importance.

Small-pox can, but probably never will be, wholly eradicated. The chief obstacle which stands in the way of its eradication is an inability to recognise facts, and to make the proper deductions from them, which seems to be associated with certain orders of mind. The facts with regard to the production of small-pox immunity by vaccinia are perfectly established. The order of mind which leads to their denial will probably never disappear from the human race.

REFERENCE.

[1] Pearce. *Boston City Hospital Reports*, 1899, p. 39.

CLINICAL OBSERVATIONS ON VARIOLA

BY

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With Seven Charts

CLINICAL OBSERVATIONS ON VARIOLA.

BY

I. R. BANCROFT.

THIS paper is based upon the personal experience of the writer as resident physician at the Boston Detention Hospital during the epidemic of small-pox in 1902-3, and presents the results of the study of about 1,200 cases.

The epidemic referred to was of moderate extent and severity. About 1,600 cases of the disease were reported, and the general mortality was 11.1 per cent. A large proportion of the sick were of Canadian birth. Individuals of all ages were susceptible, although children between the ages of five and fifteen years showed a certain degree of immunity referable to primary vaccination upon entering school. The various types of the disease were well represented.

The following classification is adopted[1] :—

(1) Variola vera and its modifications.
(2) Variola sine eruptione.
(3) Variola hemorrhagica, primary and secondary.
  (*a*) Variola pustulosa hemorrhagica.
  (*b*) Purpura variolosa.

(1)—VARIOLA VERA.

The clinical course of the ordinary form of variola includes two periods : (1) The interval between the onset of the disease and the appearance of the eruption, usually about three days; and (2) that between the outbreak of the eruption and its final disappearance, usually about two weeks. These will be referred to as the initial stage and the stage of eruption.

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[1] The writer is inclined to attribute the mildness of the disease in variola without eruption and in abortive variola vera to the absence of secondary infection.

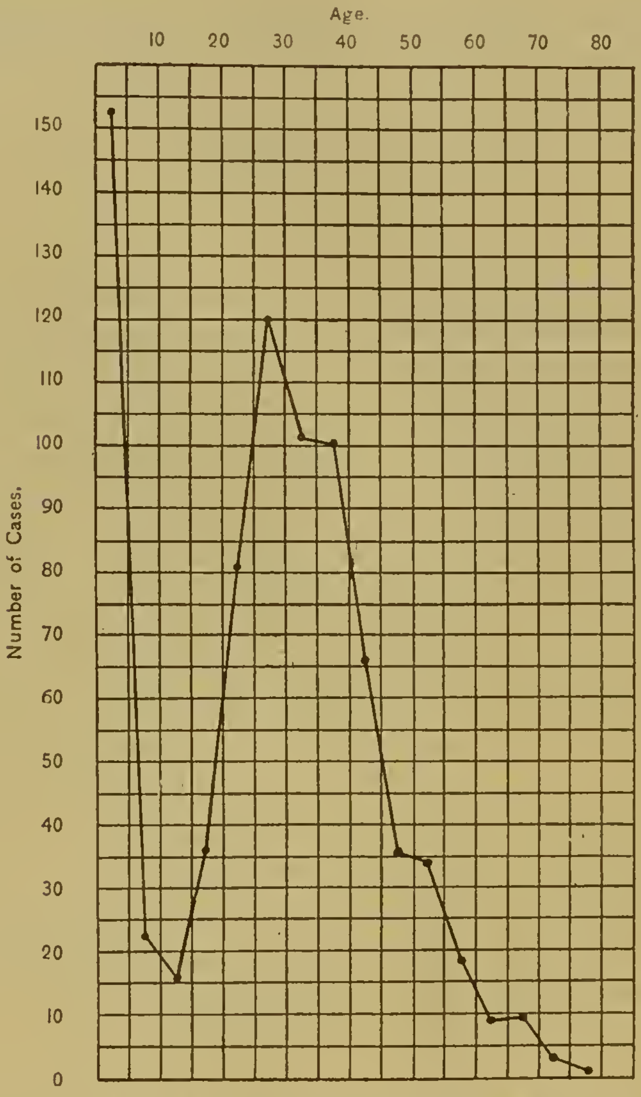

Chart 1.—A curve based upon eight hundred consecutive cases of variola, showing the occurrence of the disease at different age periods. Note the infrequency of the disease in the period coinciding with that of the school age and of compulsory vaccination.

Initial Stage.—The onset of the disease was usually sudden and marked by definite symptoms, such as headache, backache, chill, and vomiting; occasionally it was gradual and accompanied by malaise, fugitive pains, and gastric disturbances. Headache, as an early symptom, was common, usually severe, and often sharp and agonising; in some instances it was no more intense than the headache of any other febrile disease. The localisation of the pain varied; in some cases it was diffuse; in others it was referred to the frontal and to the cervical regions. The next most common symptom was backache, often acute and usually localised in the lumbar region; it was sometimes slight in degree, sometimes absent. It occurred either during the last days of the initial period or at the onset of the disease, and usually disappeared with the fall of the temperature. The pain in the back was often accompanied by pain in the thighs and the hips, and in the bones and the joints. Pain in the chest, in the epigastrium, and in the abdomen, either alone or in connection with the backache, was often present.

A chill, more or less definite in character, usually marked the beginning of fever. The temperature rose rapidly to about 104° F., and remained quite steadily at its height, with very slight morning remission, for about three days. In the lighter cases the temperature fell to normal at the end of the third day; in the more severe the decline was gradual and the return to normal not completed before the fifth or sixth days of the disease. With the fall of the temperature appeared the eruption, the full development of which was reached after normal temperature was established. In a case of moderate severity, the temperature usually reached the normal point two days after the beginning of the eruption.

The pulse and the respiration were both raised in proportion to the degree of the fever.

Gastric symptoms were commonly observed during this period of the disease, at the onset in the form of nausea and vomiting, and later as anorexia and gastric irritability, often lasting for two or three days. The tongue was usually covered with a thick yellow coat, and the breath was offensive.

Cough and sore throat occurred in a small number of cases, and chiefly during the winter months. It is probable that in many instances the presence of these symptoms was a coincidence.

Nosebleed, in cases of moderate severity, was present only occasionally, but in the more severe forms of the disease it constituted a common early symptom.

Disturbances of the central nervous system were frequently manifest. Insomnia, in part due to pain, occurred even in mild forms of the disease. Somnolence, sometimes to the extent of unconsciousness, was present, especially in children. Convulsions occurred in the cases of several children, some of them suffering from only a mild form of variola, during the initial fever. One child, with constantly recurring convulsions, was in the status epilepticus for an entire day. Nearly all patients complained of terrifying dreams, and many even tried to keep awake because of the horrible nature of their dreams. Delirium was often present, even in cases which subsequently showed a very slight eruption. It was nearly always violent and of the type seen in inebriates. Patients sometimes refused food and drink. Occasionally the most violently delirious would suddenly become rational. Others were exhausted by the violence of their delirium and died before the full development of the eruption. In two cases katatonia and sensory disturbances in the form of local anæsthesia were apparent. Great weakness and vertigo were not rare, and occasionally were mistaken for alcoholic intoxication. Syncope, even in the most rugged, was a not infrequent symptom. Disturbances of speech, exclusive of those incidental to delirium, were observed in three cases ; in one, consisting of a slight stammering ; in two, of a transitory aphasia, lasting from the third to the fifth days.

Metrorrhagia occurred frequently during the initial stage, and in one case was the cause of death. Abortion occasionally took place in this period, but more often later in the disease.

The urine at this time usually contained albumin, and presented the characteristics common to febrile diseases.

The spleen was sometimes found to be enlarged, but the enlargement was neither constant nor persistent.

The length of the initial stage was usually three days. Variations from this interval were observed in 263 out of 530 cases. In 7 the initial stage was absent; in 26 it lasted for one day; in 91 for four days; and in 139 for two days. Analysis of these cases shows that no relation exists between the length of the initial stage and the severity of the disease.

Stage of Eruption.—With the subsidence of the temperature, at the end of the initial stage, the patient was usually free from symptoms, possessing a good appetite and a retentive stomach.

The eruption was often preceded by a diffuse erythema of the face, especially of the forehead. This erythema was distinct from the initial rash (*vide* infra.).

The specific eruption varied widely in its characteristics according to the part of the body involved and the thickness of the skin. Patients ordinarily noticed it first on the forehead and on the wrists; close examination, however, usually showed a few macules on the abdomen before these regions were affected. As the most typical may be regarded the lesions occurring upon the anterior surface of the wrist at the carpal fold, where the skin is of moderate thickness. The lesion first appears as a small red macule, one to three millimetres in diameter. It is sometimes preceded by an area of slight induration. Upon pressure the macule loses colour, and there appears outside of it an area paler than the surrounding skin and resembling the blanched circle of a mosquito bite. Removal of pressure is quickly followed by return of the blood, during which may be seen in the macule a capillary pulse. The latter is more readily seen if but slight pressure be made. It is, however, often visible without manipulation. The phenomenon is demonstrable after the lesions have become well-marked vesicles.

The macule is not of uniform colour, but may be divided into an inner area of hyperæmia, surrounded by a zone of greyish colour, beyond which is a second zone of hyperæmia. The middle grey zone contains fluid within the epidermis, confined in spaces and not removable by a single prick of a needle. With the evolution of the lesion, there appears at the middle of the central red area a small grey spot, which gradually becomes filled with fluid. The grey, vesicular zone increases in size, rising above the level of the inner part of the lesion, and forming a crater or umbilication. It later becomes connected with the grey area at the centre by radial channels traversing the intervening red zone, which, for a time, shows disconnected remnants, but finally vanishes. The pock has now become a tense, hemispherical elevation, either umbilicated or rounded, containing gray, transparent fluid, and surrounded by a red areola. The fluid gradually assumes a yellowish tinge, and by the eighth day of the disease the pock is usually filled with a distinctly puriform fluid.

From this climax of development a gradual retrogression, beginning usually on the tenth day, takes place. The lesion becomes less tense and less rounded, and umbilication, due to the collapse of the centre, may appear. Its contents dry up, and it becomes converted into a solid, reddish-brown, gutta-percha-like substance. This, for a time, is closely adherent to the solid base, but later separates and falls off as a flat, hard, circular disk, of an average diameter of three millimetres.

The lesions on the face differ from the type pock above described, in that the concentric areas are in them seldom well marked, and that they present a more pronounced initial induration. The early lesion of the face is a papule, often with a shallow depression at its apex. In this region the evolution of the lesion is more rapid than elsewhere, and the pustular stage more quickly reached, the climax of development occurring on the eighth or the ninth day. By the ninth day, in all but the lighter cases, an exudate appears upon the surface of the pock, consisting of a thick, yellowish material which soon dries into a gray or greenish-gray crust. When the lesions are close together, this crust may completely envelop the face and harden into a tough brown mask. When they are discrete, the exudate in drying forms a disk upon the apex of the pock. Upon the scalp the lesions appear later than they do upon the face, and may cause great pain. Marked œdema of the scalp and of the face, leading to closure of the eyes, may appear just before pustulation.

Upon the arms many of the lesions show a region outside of the areola of lighter colour than that of the skin in general, forming a white circle, the diameter of which is about twice that of the lesion. This circle by the fifth or sixth day disappears. The eruption on the hands usually is abundant, causing an œdema which occasions great pain. The patient at this time assumes a characteristic attitude, elevating both hands and spreading the fingers as widely as possible. When the horny layer of the epidermis of the palms is thick, the pock may pass through all of its phases without distinctly elevating the surface. After the absorption of the vesicle contents, large disks remain beneath the horny layer which must be picked out before the patient is discharged from the hospital.

The eruption on the trunk is later in appearing than is that on the face or on the arms. It is usually less abundant on the abdomen and in the groins than elsewhere. It is profuse on the back, especially in its upper part, and here the lesions are generally accompanied by an extensive area of erythema surrounding the areola, forming irregularly rectangular areas, the long axis of which is parallel with the ribs. This erythema persists until the pustular stage is reached. The pocks on the body are often umbilicated, but lack the details of the concentric ring formation.

The lower extremities in general exhibit the eruption later than any other part of the body, although a small area of pock formation may appear very early upon the inner aspect of the thigh. The lower leg is often almost wholly free from eruption.

The drying of the lesions ordinarily begins before the decline of the temperature. Those on the face dry first, next those on the arms, later those on the trunk and the lower extremities. The crusts of the individual lesions fall off, and sometimes the epidermis of the palm or the sole is cast off in one piece, carrying upon its inner surface disks. When the lesions are deep, scars usually result. In some severe cases the nails fell off. In nearly all cases with secondary fever, the hair fell out during convalescence.

The eruption on the mucous membranes is typified by that on the soft palate. It begins here, before the appearance of any lesions upon the skin, with the formation of a papule. This papule develops into a vesicle which ruptures, forming a white, crater-like erosion. In this region, the eruption is most abundant upon the soft palate, although the entire mucous membrane of the fauces may be affected.

The pocks may pursue a course of development unlike that described as typical. One of the most common variations, seen in severe cases of the disease, is delay in the evolution of the eruption, whereby the pocks, which in lighter cases are well filled out and pustular by the eighth day, do not reach their full development before the twelfth day.

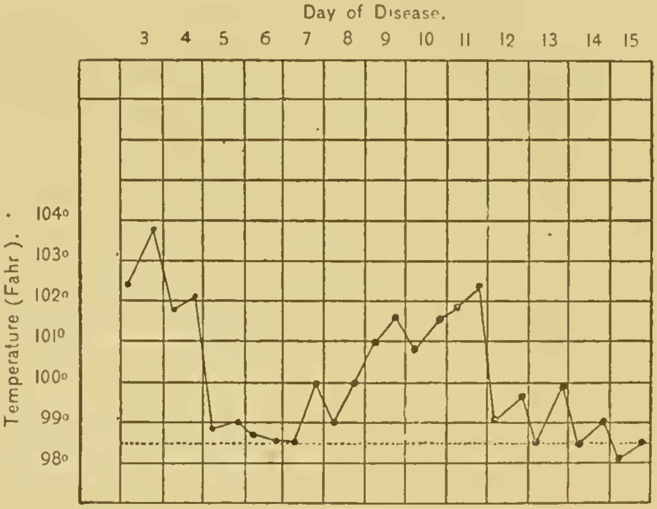

Chart 2.—Variola vera, typical case.

Another atypical condition is seen occasionally toward the fatal termination of the disease. Instead of developing into full, rounded pustules, the pocks may remain umbilicated or even flatten down, puckering in the centre, and presenting a dull gray instead of a glistening yellow colour.

Not infrequently the centre of the pock becomes dark red from hæmorrhage. This hæmorrhagic condition is seen mostly in the lesions of the forearms and of the legs.

These atypical pocks show less than the ordinary degree of peripheral inflammatory reaction.

An eruption which has shown atypical characteristics during the first week of the disease may later become typical; the reverse of this is also true.

The usual development of the pocks upon the trunk may further be modified during the vesicular stage by the formation of impetiginous lesions about a centimetre in diameter, which remain flat. In other cases the pocks are merged into superimposed bullæ, often of large size, which contain a fluid, nearly clear, and so continuing.

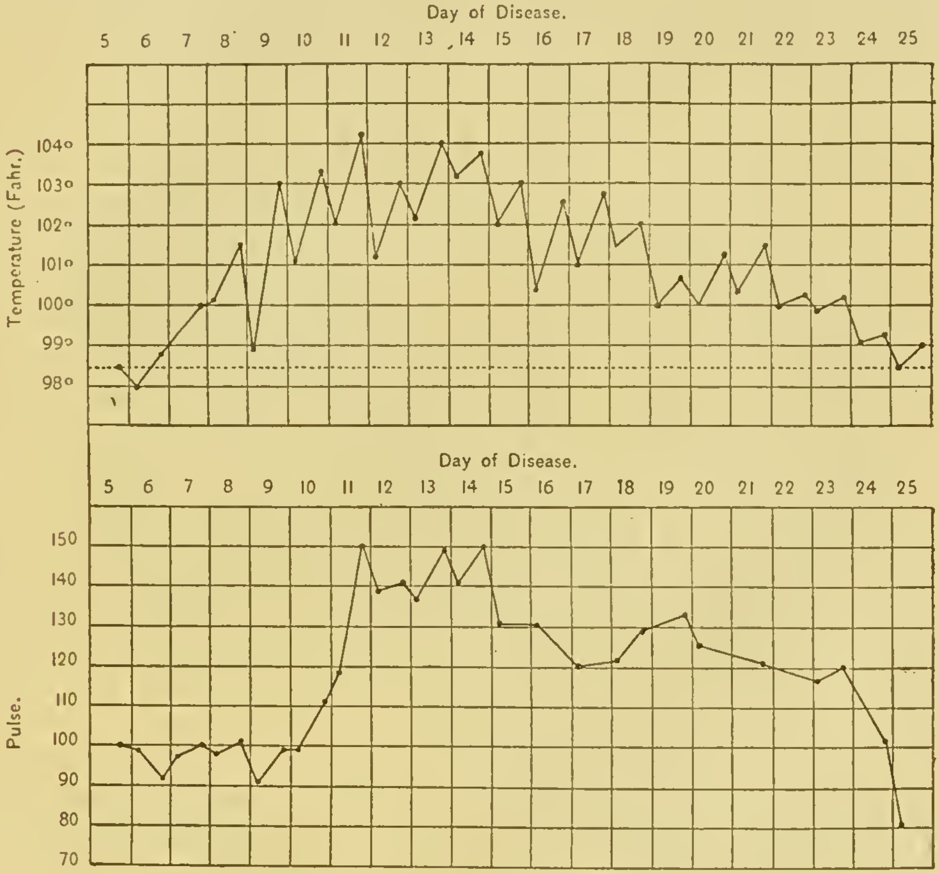

CHART 3.—Variola vera, severe.

The pock contents may become mixed with blood, or the areola assume a purpuric character, conditions often seen in the eruption on the lower extremities.

At times, in the place of ordinary vesicles, the earlier lesions consist in purpuric macules, which may either develop into the usual pustule surrounded by a purpuric areola, or remain as small, bright red spots.

The temperature, normal at the beginning of the stage of eruption, usually so remained for three or four days. It then gradually rose, often without morning remission, reaching a maximum of 102° to 104° F. by the twelfth or thirteenth day of the disease. In the more severe cases the temperature did not return to normal at the end of the initial stage, but declined only two or three degrees, rising again on the sixth or seventh day of the disease.

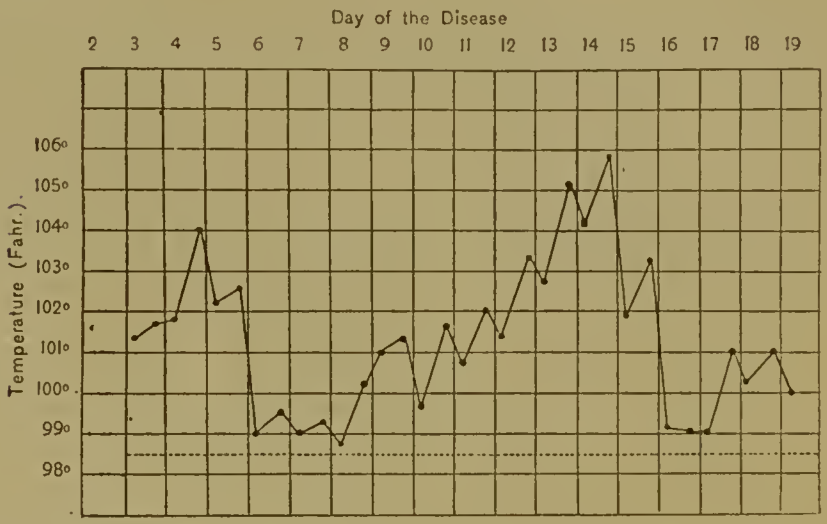

CHART 4.—Variola vera, severe, with crisis on the fourteenth day of the disease.

The following table shows the maximum temperature in 200 cases of variola vera, one half of which were fatal :—

Temp. (Fahr.)	Number of cases.	
	With recovery.	Fatal.
100°	2	1
101°	17	6
102°	37	16
103°	27	21
104°	16	25
105°	1	21
106°	..	8
107°	..	2
Total cases	100	100

The character of the fever is of some value in prognosis. The earlier the temperature reaches its maximum the lighter is likely to be the form of disease. Persistence of the fever throughout the period of the initial stage forecasts a severe form of the disease, while its early subsidence means the contrary.

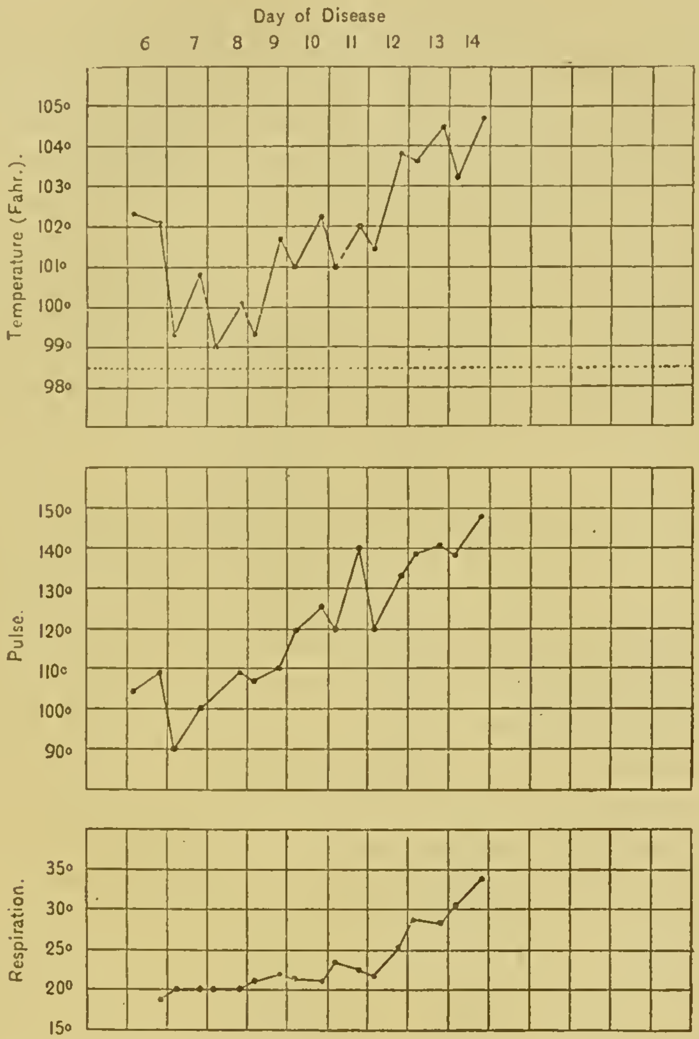

CHART 5.—Variola vera, fatal on the fourteenth day of the disease.

The decline of the temperature was usually gradual. After continuing at its height for two or three days, in case of recovery it slowly fell, sometimes not returning to the normal until the fourth week of the disease.

The cases were rare in which the temperature dropped from its maximum to the normal in twenty-four hours.

The pulse rate, which during the afebrile period was often perfectly normal, followed the temperature in its rise, and sometimes, even in cases with recovery, exceeded a rate of 150 to the minute.

Disorders of the respiratory system, other than those occurring as complications, were not common, but when present consisted of cough, dry, sometimes severe and persistent, and occasionally accompanied by bloody or rusty sputum. Obstruction of the larynx, necessitating intubation, occurred in three cases, all children under five years of age.

Disturbances of the digestive system were infrequent. Rarely there was slight nausea. Sufficient nourishment could generally be taken throughout the stage of eruption. Glossitis was seen in a few cases, in one of which it was so severe as to demand recourse to nasal feeding. Occasionally there was a profuse secretion of saliva.

Disorders of the central nervous system were seen at this stage of the disease rather more frequently than during the initial stage. Delirium, in inebriates often of violent character, appeared on the fourth or fifth day, and such individuals frequently died before the disease had reached its climax. Unconsciousness was common in severe cases during the last three or four days of life, or, in such as recovered, for an equal period prior to the climax of the disease. On the other hand, some patients having a severe and fatal form of the disease retained consciousness throughout.

Albuminuria was present in severe cases at the height of the secondary fever, and occasionally during convalescence.

Nodules in the testicles, small and hard, were present in certain severe cases at the pustular stage of the eruption.

Complications.—The complications of variola vera are numerous and varied.

Toxæmia, other than that incident to local secondary infection, was evidenced in certain cases by a rise of temperature on about the sixteenth day.

Secondary infections of the skin were frequent during the late stages of the disease in the form of furuncles, small abscesses, erysipelas, and cellulitis. These conditions occurred upon any part of the body. Purulent inflammation of the elbow-joint was seen in the third week of the disease in two cases. Acute non-suppurative arthritis was common.

Conjunctivitis was nearly always present. Pock formation leading to ulceration was seen upon the conjunctiva, but, save in one case of pannus, was not observed upon the cornea.[1] Lesions at the limbus often extended to the cornea, and gave rise to ulcerative keratitis. Ulceration of the centre of the cornea, without previous lesions, was seen late in the disease.

Disorders of the respiratory system were common complications. Broncho-pneumonia was frequent in fatal cases; lobar pneumonia was rare, but occasionally developed after convalescence seemed to be established.

Pleurisy and empyema were uncommon. Bronchitis was so frequent as to justify regarding it as a symptom rather than as a complication. Œdema of the larynx was occasionally present, and in many cases there was aphonia.

Inflammation of the salivary glands was frequent, and occurred usually in the third week. The parotid gland was affected most often. In the absence of suppuration there was a profuse secretion of saliva.

Suppurative parotiditis was a frequent and sometimes fatal complication. Noma and local gangrene following this condition were seen in three cases.

Inflammation of the middle ear was a rare complication, present chiefly in children.

Endocarditis was uncommon. Impairment of the action of the heart was usually referable to degenerative myocarditis or to anæmia.

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[1] The case here referred to was that of a woman who gave a history of definite exposure to variola on October 20 from 3 until 10 p.m., and of onset of the disease on October 30 at noon. The eruption appeared November 1. Old keratitis, with pannus, was present in both eyes, more marked in the left. Upon the vascularised portion of the left cornea occurred a typical papule, which underwent the evolution characteristic of the specific lesion upon a mucous membrane.

Disorders of the genito-urinary system were infrequent. Acute nephritis was rare. Suppurative orchitis was not observed.

Abortive Types.—Under this head are included forms of small-pox in which the course of the disease, both as to constitutional symptoms and as to eruption, is of much less than ordinary severity, and which are generally designated as "varioloid."[1]

The onset in such cases may be marked by very indefinite symptoms, such as slight headache or pain in the back, initial fever being unnoticeable or absent. In some instances an initial fever of the most severe type was present, accompanied by delirium and unconsciousness, and continuing for four or five days. More commonly the initial fever was of short duration.

The eruption in cases of this sort was of varied character. In many the lesions at the onset were numerous, closely-set, but finer and more superficial than usual. The vesicle developed on the apex of the papule, never completely replacing it, and on the fifth or the sixth day, instead of becoming a full, tense pustule, collapsed and dried up, forming a thin brown crust which was soon desquamated. This modification of the ordinary evolution was seldom manifested by all of the pocks upon the body; more commonly, certain lesions continued to develop into fully-formed pustules, so that scattered among the abortive pocks were occasional typical pustules. The lesions of the lower extremities showed the least tendency to modification. The eruption in certain cases was rendered yet further atypical by the development of purpuric areolæ about the lesions, or by the appearance of purpuric macules among the pocks.

Another modification of the eruption consisted in sparseness of the pocks, which, notwithstanding their small numbers, presented a natural evolution. In certain cases of this type the individual lesions were of very large size. In such, the course of the disease was sometimes fairly severe, although unaccompanied by secondary fever.

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[1] *Note.*—This term is sometimes restricted to those cases in which the disease is modified by previous vaccination.—Ed.

In a few instances the pocks were larger than the ordinary and presented a solid base, the latter persisting as warty protuberances long after the disappearance of other evidences of variola.

A further modification of the eruption was characterised by the appearance of successive crops of lesions. In cases showing this modification, a sparse eruption, becoming pustular by the fifth day of the disease, developed first upon the face, and was followed by the appearance of lesions upon the other parts of the body on the third, the fourth, and the fifth days. This resulted in the co-existence of pocks at all stages of development, the earliest lesions being dry by the time the later had become pustules. The vesicles in such cases were very superficial, and those upon the arms could easily be broken by the pressure of the finger. In this form of the disease no symptoms were present after the subsidence of the initial fever, and complications were rare.

(2) Variola Sine Eruptione.

Illness of indefinite character, appearing on the twelfth day after exposure, was seen in hospital attendants and in others exposed to infection. The symptoms, lasting for two or three days, consisted of headache, pain in the back, fever and nausea. After the subsidence of the fever, a few definite pocks occasionally appeared. These conditions were observed in four physicians and in eight attendants.

Incidents in the course of the epidemic warrant the belief that variola without eruption and unrecognised was not infrequent. Inquiry at the time of the removal to hospital of a patient with well-marked small-pox, often brought to light the fact that about two weeks previously another member of the household had been sick, who, upon examination, frequently showed a few healed pocks. In one such case, the crust of the "pimple" from the suspected individual gave the characteristic corneal reaction in the rabbit. One patient, who had a well-marked initial fever accompanied by a typical initial rash, subsequently presented no eruption whatever. Another, a pregnant woman, remembered having a slight headache after exposure to the disease, but was not otherwise ill. Her child showed a typical eruption when two days old. Cases of this sort were doubtless the cause of outbreaks of the disease in hospitals and in other institutions. A group of three cases in one of the large hospitals in Boston, the onset in whom was nearly simultaneous, was traced to a ward tender who had had an attack of what was supposed to be the " grip."

(3) Variola Hæmorrhagica.

The hæmorrhagic varieties of small-pox differ widely from the other forms of the disease. They are classified according to the relation of the purpura to the specific eruption. The form in which the purpura precedes the specific eruption is known as purpura variolosa, or primary hæmorrhagic small-pox ; that in which the purpura follows a pre-existing specific eruption is designated as variola pustulosa hæmorrhagica, or secondary hæmorrhagic small-pox.

(*a*) *Variola Pustulosa Hæmorrhagica.*—In the course of the epidemic, this form of the disease was frequently observed. It occurred usually in young adults, and was seen in no one under 20 years of age. Its occurrence bore no determinate relation either to sex or to habits.

The onset, which in some cases was preceded by malaise and by indefinite pain, was usually marked by severe symptoms. The initial fever was high, but the course of the disease in the initial stage was not essentially peculiar, save that at its end in severe cases there was no fall of temperature. In such the purpuric conditions were first evidenced by hæmorrhage into the areolæ of the pocks. In some instances the hæmorrhagic tendency was first indicated by persistent nosebleed, and by hæmorrhage from the conjunctivæ, and from the gums ; and in yet others by hæmaturia and by metrorrhagia. A condition was seen simulating hæmophilia, in which slight accidental wounds bled persistently.

In less severe cases the hæmorrhagic condition appeared in the vesicular stage of an eruption developed in the usual position after a severe and protracted initial fever. The hæmorrhage showed first on the lower extremities in the centres of the pocks, filling them with bloody fluid, a condition often coincident with hæmorrhages from the mucous membranes.

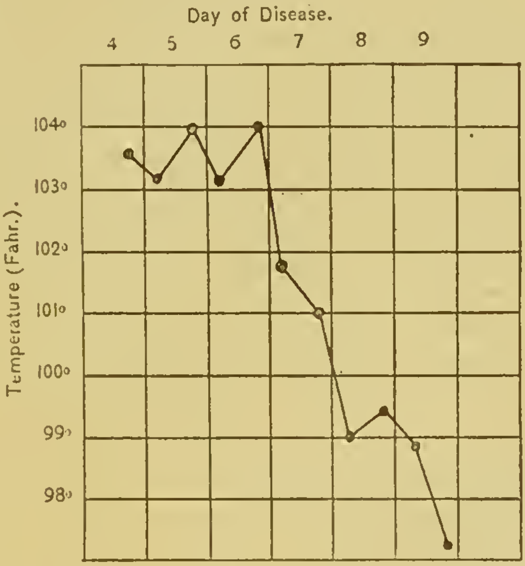

Chart 6.—Variola pustulosa hæmorrhagica.

Patients usually lived for several days following the appearance of the hæmorrhages, which allowed for the development of pustules. Although some lesions showed an admixture of blood and pus, the exudate was never sufficient to cause complete filling out of the pocks.

In cases with recovery the pustules of the lower extremities sometimes contained a little blood, and occasionally there was nose bleed. Patients showing well-marked hæmorrhagic tendencies invariably died, others in whom this tendency appeared late in the disease, and was of slight degree, recovered.

(*b*) *Purpura Variolosa*.—In this form of variola a general erythema precedes any other outward sign of the disease; this and other characteristics distinguish it sharply from variola vera, and from other types of small-pox.

The occurrence of purpura variolosa varies widely in different epidemics. In that of 1870 it was frequent; in the recent outbreak it was present in 1 per cent. of 1,200 cases.

The relation of this type to other forms of the disease was wholly indeterminate. In two instances it followed exposure to mild forms of variola vera. In no instance did one case of the purpuric form give rise to another. Cases of variola traceable to purpura variolosa as a source of infection showed no uniformity in type.

Relative to vaccination, analysis of the twelve cases showed that recent vaccination had been practised in none, although in two unsuccessful attempts had been made within the two weeks preceding the onset of the disease; that primary vaccination in childhood had been attempted in seven, of which only three, men of an average age of 27 years, showed typical scars; and that three were unvaccinated.

As to sex, ten of the cases were men, and two women; neither of the latter was pregnant.

The age limits were twenty-four and fifty-one, with an average of thirty-four years.

Alcoholism as a factor in predisposition was of doubtful importance. Five individuals used liquor to some extent; of the three of these who showed typical scars of vaccination in childhood, two were markedly intemperate.

The period of incubation could in no case be exactly determined.

The onset, preceded by prodromal symptoms of indefinite character, differed but little from that of variola vera. The temperature was not as high as in the latter form of the disease, and their history showed that patients had in several cases worked during the first two days of the initial stage, in two instances at hard labour.

On the second day an erythema, superficial, bright red, and resembling that of the initial rash, appeared on the chest or on the arms, which within twenty-four hours overspread nearly the whole body, and was accompanied by marked increase in the severity of the constitutional symptoms. Pain, severe, deep-seated, and agonising, was present in many parts of the body, including the bones and the joints, but was most intense in the præcordium and in the epigastrium. Pain in the back was not a prominent symptom.

The temperature was rarely high, usually less than 103° F. The pulse was rapid, reaching 160 to the minute. The respiration was rapid and laboured without evidence of organic disturbances in the lungs. Cough was present and persistent, accompanied by the expectoration of fluid consisting almost wholly of blood. The tongue became swollen and covered with dark crusts of dried blood and mucus. Nausea and vomiting were persistent, the vomitus often consisting almost wholly of blood.

The erythema, first apparent on the second day of the disease, on the third day changed from bright red to a bluish terra cotta colour. This change was most marked on the face and on the extremities. At the same time the skin became mottled with petechiæ. These were of two sorts: one, a small, round, or irregularly-shaped area, usually less than two millimetres in diameter, bright red in colour, superficial, and widely distributed, although most numerous on the face, on the extremities, and on the abdomen; the other, somewhat larger and more deeply seated than the above, from two to fifteen millimetres in diameter, of irregular contour, plum coloured, and most numerous on the eyelids and on the alæ nasi.

In addition to the erythema, and to the petechiæ, many of the cases presented a few small superficial gray vesicles, containing a small amount of clear fluid, and most frequently seen on the face and on the body. In some instances a dozen or less of these rudimentary vesicles were the only evidence of the specific eruption. In others even these were absent.

Individuals who lived beyond the fourth day of the disease usually showed the beginning of a typical small-pox eruption, with lesions on the palms and on the soles, but rarely elsewhere. At this time the petechiæ increased in number and the erythema grew yet darker. With the progress of the disease, hæmorrhage from the mucous membranes became more and more marked. Bleeding from the stomach, the intestines, the kidneys and the bladder was common. All of the women suffered from severe metrorrhagia. Dark crusts of dried blood formed in the nares and in the mouth, from beneath which blood slowly oozed. The gums became red and soft, and bled easily. The breath was extremely fœtid, and the tongue was swollen, dry, and crusted,

The urine was scanty, high-coloured, and bloody, and contained blood casts in small numbers.

The sensorium was surprisingly clear; the patient was at times drowsy, but could always be awakened to complete consciousness, and was able to converse intelligently up to the time of his death.

Death always took place within a week from the onset; the average duration of the disease was five days.

Initial Rashes.—During the initial stadium there may appear upon the skin an erythematous or a petechial rash, lasting from a few hours to several days, and bearing no resemblance to the specific eruption. In many instances the two forms of rash were associated.

The erythema was observed less frequently in this epidemic than it has been in others. Of sixteen cases of initial rash, the pure erythematous type occurred in but six, and in these the variola was of mild form, without secondary fever. The rash usually appeared on the second day of the disease, reached its full development on the third day, and gradually faded, finally disappearing before the development of the specific eruption. It was sometimes prolonged into the stadium of eruption for a few hours, or even several days, but in such cases usually did not persist beyond the late vesicular stage of pock formation.

The distribution of the rash varied from a mere flushing of the face, frequently seen during the last day of the initial fever, to a general erythema, a condition seen more rarely, in which the skin of the entire body was of a bright red colour. The erythema of the lower extremities was often faint, and, even when elsewhere intense, was here patchy and coarsely mottled with areas of normal skin. In one instance the rash was composed of fine macules; this bore no relation to the specific eruption, and lasted for twenty-four hours.

The coincidence of the erythema with the eruption gave rise to yet other conditions, in general characterised by the presence of large areas of erythema surrounding or in the vicinity of pocks, and developed by the extension of the areolæ. These areas, often four or five centimetres in diameter, were irregularly oval, and were most numerous upon the back, parallel with the ribs. In children large areas of this sort, eight to ten centimetres in diameter, and widely distributed, occurred without relation to pocks.

The petechial form of rash was seen in ten cases. It seldom occurred unassociated with erythema, and even when well-marked often appeared as a diffuse, dark red erythema, which only upon pressure disclosed petechiæ, bright red, round or irregularly oval, and from one to two millimetres in diameter.

When not general, this rash was most commonly present upon the abdomen, within an area below the umbilicus, shaped like the wings of a butterfly, a region usually comparatively free from pocks. This fact has suggested the possibility of a relation between the presence of the initial rash and the absence of the specific eruption. In one instance in this series of cases initial rash in the region designated was followed by an abundant eruption of pocks.

In the less marked cases the rash occurred in detached patches situated usually in the folds of the skin at the axilla, the groin, and, less commonly, at the elbow and the knee. These areas were most extensive when the petechial rash was accompanied by erythema than when it occurred alone.

The petechiæ were numerous in scar tissue, notably in the striæ albicantiæ of the abdomen.

Of ten cases in which this form of rash was well-marked, four exhibited a mild form, two a severe form, and two a fatal form of the disease.

The duration of the petechial rash was somewhat larger than that of the erythema. Its appearance changed rapidly after the third day, when the associated erythema disappeared, and the petechiæ gradually became yellowish-brown, and at the end of the week generally disappeared.

This rash may closely resemble that of purpura variolosa; both appear at the same stage of the disease, and at first look very much alike. Later, the erythema of purpura variolosa assumes a characteristic, dark, terra-cotta-red colour, and is often further differentiated by the presence of ecchymoses in the deeper layers of the skin and beneath the mucous membranes.

Secondary Rashes.—Another type of rash, developed late in the disease, within or following the period of crust formation, was observed in a small number of cases, less than one per cent. of the whole series, and with one exception occurring within a space of three months, near the close of the epidemic.

This secondary rash exhibited two forms: (*a*) a fine, red maculo-papular eruption; and (*b*) a mottled erythema. The first mentioned followed light cases of variola, was present chiefly on the body, rarely on the arms, hands, and face, and was unaccompanied by itching. It appeared soon after the climax of the secondary fever, and lasted for about ten days. Coincidentally with it occurred fever and usually headache, nausea and vomiting. In some instances the rash was present without these symptoms; in others they occurred without the rash.

The second form of secondary rash, the mottled erythema, was seen more frequently than the first. It occurred in the course of the more severe types of variola and was always preceded by constitutional symptoms, which usually appeared on the thirteenth day and consisted of fever, in seven cases amounting to 104° F., headache, nausea and vomiting. The eruption commonly appeared either with the rise of temperature or on the day following, and closely resembled that of scarlet fever. In the more severe cases the erythema was general, in the lighter it was localised on the abdomen, on the flexor surfaces of the arms and on the legs, and in the folds of the axillæ and of the groins. Its duration was ordinarily from two to three days and it was followed by desquamation. In several of the cases there was subsequent rise of temperature, occurring repeatedly, and with some suggestion of periodicity. In two cases there was an associated suppurative cellulitis in the region of the elbow. One case died on the twenty-ninth day of the disease (see chart No. 7).

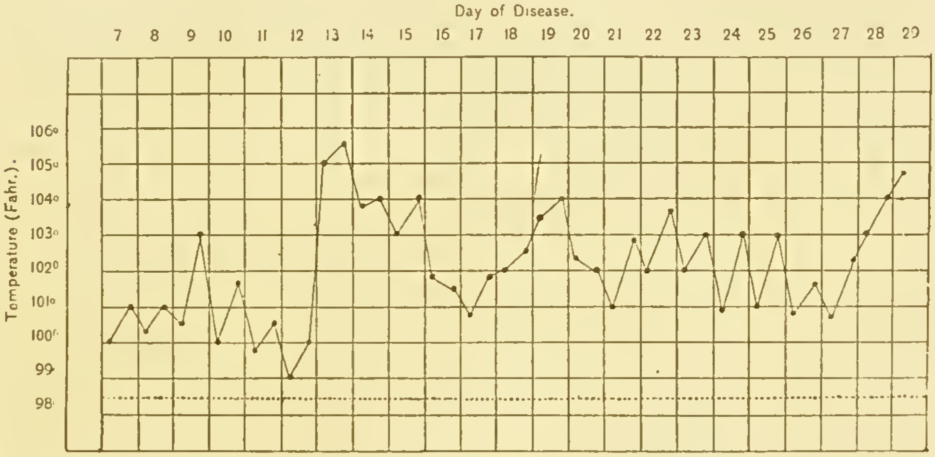

CHART 7.—Variola vera, light case, with secondary rash; death on the twenty-ninth day of the disease.

The condition was first regarded as scarlet fever and patients suffering from it were isolated. One such, who exhibited a rash on the ninth day of the disease, accompanied by fever on the ninth, the twelfth, and the fifteenth days, was discharged from the hospital on the twenty-fifth day with desquamation incomplete. Eight days later a member of his family was admitted to the scarlet fever wards of the Boston City Hospital, with no known source of contagion. On the other hand the writer, who was constantly exposed to cases of this sort, six months later, after the conclusion of his services at the hospital, acquired scarlet fever.

THE

FORTY-SIXTH ANNUAL REPORT

OF THE

NEW SYDENHAM SOCIETY

WITH

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Those marked with an asterisk were not in office last year.

REPORT.

PRESENTED TO THE FORTY-SIXTH ANNUAL MEETING OF THE NEW SYDENHAM SOCIETY, HELD AT LEICESTER, FRIDAY, JULY 28th, 1905.

THE year's issue has comprised as usual four Fasciculi of the Clinical Atlas. Amongst the subjects which chiefly have been illustrated are the Eruptions caused by Iodides and Bromides, Lichen Urticatus (concerning which the question has been opened as to its possible causation by insect bites), Pemphigus, Leucoderma, Myxœdema.

The Council has again to express its regret that the funds at its disposal have not allowed of the publication (in addition to the four Fasciculi of the Atlas) of a printed volume. In the hope of attracting new members, and in the desire at the same time to increase the privileges of existing ones, it has been decided to allow all members to select from the back stock, without payment, one volume every year. This stock comprises some of the most valuable of the Society's publications in the past. It is hoped that members will do their best to make known amongst their friends the advantages thus offered, and will endeavour to enlist new members. A list of the works available can be obtained on application to the Society's agent.

The Council has pleasure in announcing that there is a good prospect that the income for the current year will allow of the publication of a printed volume. This will consist of Selected Essays, taken chiefly from German sources. It will include, with many others, the most recent observations on the parasite of Syphilis.

The Society's accounts for the year ending Dec. 31st, 1904, have been audited as usual. A balance of £65 13s. was then in the Treasurer's hands. The items of expenditure have been very carefully controlled by the Council. In connection with the endeavour to make the Atlas more widely known a larger sum than usual has been spent in advertisements. The apparent excess in this and other details of the Society's depôt as disclosed in the present balance-sheet is, however, due the fact that certain cheques for sums due in the preceding year did not reach the banker's hands until after its close, and could not therefore be included in last year's statement.

Dr. THE NEW SYDENHAM SOCIETY — BALANCE SHEET FOR 1904. Cr.

RECEIPTS.

	£	s.	d.	£	s.	d.
Subscriptions— 1 for 1893	1	1	0			
,, 1 ,, 1896	1	1	0			
,, 1 ,, 1897	1	1	0			
,, 1 ,, 1899	1	1	0			
,, 3 ,, 1900	3	3	0			
,, 73 ,, 1901	76	13	0			
,, 162 ,, 1902	170	2	0			
,, 348 ,, 1903	365	8	0			
,, 732 ,, 1904	768	12	0			
,, 67 ,, 1905, paid in advance	70	7	0			
1,389 Subscriptions	1,458	9	0			
Back Volumes	37	19	2			
Repayment of Postages, &c., charged in Agent's Disbursement Account	49	6	6			
	1,545	14	8			
Less deductions by Local Secretaries ..	14	14	4			
				1,531	0	4
Balance at Bankers, January 1st, 1904				814	9	5
				£2,345	9	9

EXPENDITURE.

The following seven liabilities, which were mentioned in a note appended to the last Balance Sheet, were incurred in 1903, and were paid in January, 1904.

		£	s.	d.
1.	Assistant Secretary	53	10	0
2.	Secretary's disbursements (Burgess and Advertising)	15	7	6
3.	West, Newman & Co., Lithographic draughtsmen ..	237	19	0
4.	Bale, Sons & Danielsson, Lithographic printers ..	97	15	9
5.	H. K. Lewis—Agent's Salary and Commission ..	99	3	5
	,, Disbursements	49	9	7
	,, Advertisements	284	12	0
6.	Bale, Sons & Danielsson—Fasciculus 18	307	2	0
7.	H. K. Lewis—Agent's Commission	59	14	1
	,, Salary	52	10	0
	,, Disbursements	73	8	1
	,, Advertisements	69	4	6
	,, Balance of Account, 1903	4	4	10
8.	Bale, Sons & Danielsson—Fasciculus 19	277	1	6
9.	West, Newman—Twenty Chromo Atlas Plates ..	350	0	0
11.	Adlard's	46	4	6
10.	Assistant Secretary	52	10	0
12.	Mr. Lewis	150	0	0
		2,279	16	9
	Balance at Bankers, December 31, 1904	65	13	0
		£2,345	9	9

Examined, compared with the vouchers, and found correct, the Balance of the Account in favour of the Society being £65 13s. at an audit held this May 6th, 1905.

LEONARD W. SEDGWICK, } *Auditors.*
WAREN TAY,

THE NEW SYDENHAM SOCIETY.

The Atlas of Clinical Medicine, Surgery and Pathology, was commenced in 1901 and is now (1906) in its sixth year of issue. It is published in fasciculi and at least thirty-two plates are issued yearly. Its contents since its commencement are briefly noted overleaf.

The fasciculi of the first volume of the Atlas, comprising the issues for 1901, 1902 and 1903 with index, may be obtained by members for the reduced sum of Two Guineas.

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One Guinea per annum entitles members to the fasciculi of the Atlas and any other publication that may be issued for that year; also to the selection of one volume from the Society's surplus stock. A subscription of **five guineas** entitles to six years' membership and to the immediate selection of six volumes.

In addition members may purchase books from the surplus stock at the rate of seven for One Guinea.

Notice to Members Residing Abroad.—To cover the cost of postage and packing an additional 2s. 6d. or 3s. 6d. has to be added to the annual subscription, or to the charge for sets of seven volumes, the higher rate including special packing in linen for the better protection of the volumes in transit through tropical climates.

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CONTENTS OF THE ATLAS OF CLINICAL MEDICINE, SURGERY AND PATHOLOGY.

1901.

FASCICULI I. and II.—Double number. Frambœsial Syphilis (Yaws and Parangi), with descriptions and a *résumé* of present knowledge by Jonathan Hutchinson, F.R.C.S.

Plate A.—The primary sore in Yaws and in Syphilis.
,, B.—Early secondary Frambœsial eruptions.
,, C.—The eruption of Yaws.
,, E.—Late secondary Frambœsial eruptions.
,, F.—Tertiary ulceration in Yaws.
,, G.—Multiple Dactylitis, &c.
,, H.—Dactylitis, Phagedæna, and Gummata in Yaws.

Parangi in Ceylon, with descriptive remarks by Sir William Kynsey.
Plates 76-80.—The eruption.
Plate 81.—Eruption in mother and child.
,, 82.—Eruption on breasts of nursing mother.
,, 83-84.—The eruption.
,, 85.—Studies of lesions.
,, 86.—Eruption affecting the sole of the foot.
,, 87.—Studies of the lesions.
,, 88.—Ulcer of lip.
,, 89.—Ulcerations and Periostitis. Multiple Dactylitis.
,, 90.—Scar resulting from Serpiginous ulceration.
,, 91.—Scars, ulcers and dactylitis.

FASCICULI III. and IV.—Double number. Xanthelasma and Xanthoma, with especial reference to their association with functional and organic diseases of the Liver.

Plate A.—Long persisting Xanthoma diabeticorum in a woman.
,, B.—Eruptive Xanthoma in a boy, with Xanthelasma of eyelids.
Plates C, D and E.—Eruptive Xanthoma of diabetics.
,, F, G and H.—Xanthoma of palms; in Plate H associated with persistent jaundice.
,, I to M.—Xanthoma tubercles in various sites
Plate 92.—Eruptive Xanthoma in a child.
Plates 93-95.—Eruptive Xanthoma of diabetics.
,, 96-97.—Xanthoma of persisting Jaundice.

1902.

FASCICULUS V.—Coxa Vara, with Essay by C. R. B. KEETLEY, ESQ.

PLATES A and B.—Coxa Vara.
,, C to I.—Radiographs of cases of Coxa Vara.
Miscellaneous Plates :—
PLATE J.—Radiograph of ununited fracture of surgical neck of humerus.
,, K.—Radiograph of elbow joint with displacement of bones.
,, L.—Multiple Carcinomata of Skin.

FASCICULI VI. and VII. Double Number. Xanthelasma and Xanthoma; Changes in Skin caused by Arsenic; Pemphigus and its variants; Fractures and Dislocations; Miscellaneous.

PLATE 98.—Chancres from tattooing.
,, 99.—Fungating growths of melanotic sarcoma on palm and sole.
,, 100.—Xanthelasma palpebrarum.
,, 101.—Xanthoma diabeticorum.
,, 102.—Xanthoma with persisting jaundice.
,, 103.—The comedonous form of Xanthoma.
,, 104.—Arsenical Keratosis.
,, 105.—Comedonous Xanthelasma.
PLATES 106-107.—Keratosis of palms and Epithelial Cancer from Arsenic.
PLATE 108.—Keratosis of Palms from the medicinal use of arsenic.
PLATES 109-110.—Conditions resembling Psoriasis from medicinal use of arsenic.
,, 111-112.—Arsenical Keratosis of palms and soles.
,, 113-114.—Epithelioma from medicinal use of arsenic.
PLATE 115.—Keratosis of soles from arsenic.
,, 116.—Pigmentation of abdomen from arsenical poisoning.
,, 117.—Arsenical pigmentation and keratosis of the sole.
Miscellaneous :—
PLATE A.—Bromide eruption in a child.
,, B.—Illustrations of inherited syphilis.
,, C.—Impetigo contagiosa.
,, D.—Scabies, &c.
PLATES E to G.—Illustrations of Pemphigus.
PLATE H.—Dentigerous Cyst.
,, J.—Epitheliomatous ulceration in scars.
PLATES K to O.—Radiographs of various fractures and dislocations.

1902 and 1903.

FASCICULI VIII. and IX.—Double number. Arsenical Eruptions; Urticaria Pigmentosa; Leprosy.

Results of arsenical poisoning:—
PLATES A and B.—Pigmentation, Œdema, Erythema, &c.
PLATE C.—Œdema, Pigmentation, Keratosis, &c.
,, D.—Pigmentation.
,, E.—Muscular Paralysis.
,, F.—Bullous eruptions.
,, G.—Paralysis of diaphragm and extremities.

Urticaria Pigmentosa, with remarks by JONATHAN HUTCHINSON, Esq.

PLATES 118-121.—Coloured plates.
,, E. to H.—Uncoloured illustrations.

Leprosy:—

PLATES 122-123.—Diffuse Dermatitis in stage of resolution.
PLATE 124.—Leprosic neuritis.
,, 125.—Diffuse Dermatitis with swelling of external ear.

PLATES I to Z (uncoloured) shew the following features:—
The macular stage of leprosy; vesications, ulnar nerve paralysis, neuritic leprosy in late stage, paralysis of both facial nerves, perforating ulcers of feet, early stage of tuberous form, advanced stages of tuberous form, ulnar paralysis with mutilation, receding stage of tubercular form, fusiform enlargement of nerves, &c.

1903.

FASCICULI X. and XI.—Double number. Illustrations of Herpetiform Morphœa. (Coloured.)

PLATE 126.—Morphœa on arm and face of young man.
,, 127.—Morphœa on back of young girl.
,, 128.—Morphœa patches under the breast.
,, 129.—Morphœa in bilateral zones.
,, 130.—Morphœa in bilateral zones.
,, 131.—Morphœa in group on clavicular region.
,, 132.—Morphœa in temporo-malar region.
,, 133.—Morphœa in patches on face and neck.
,, 134.—Morphœa on face.

Uncoloured:—
PLATE A.—Morphœa involving almost the entire surface.
,, B.—Morphœa affecting the foot.
,, B (*bis*).—Morphœa affecting the short saphena nerve.
,, C.—Morphœa followed by hemiatrophy of the face.
,, C (*bis*).—Hemiatrophy of face.
,, D to I.—Hemiatrophy.
,, J.—Herpetiform Morphœa affecting one lower extremity.

Radiographs illustrating Colles' Fracture, with introductory remarks.

PLATES K to S.—Illustrating different varieties and stages.

FASCICULUS XII.—Miscellaneous, and Radiographs of Fractures and Dislocations.

PLATE A.—Sprengel's shoulder.
,, B.—Paralysis of serratus magnus. (Two plates.)
,, C.—Cretinism.
,, D.—Albinism.
,, E.—Bazin's Disease.
,, F.—Accessory Thyroids.
,, G.—Tuberculous Leprosy.
,, H.—Malignant Syphilis.
,, H (*bis*).—Rupial eruption in Secondary Syphilis.

,, H 3, 4, 5.—Malignant Syphilis of Frambœsial type
,, I.—Primary Chancre on hand of midwife.
,, J.—Syphilitic Serpiginous Lupus.
,, K.—Corymbiform syphilitic eruption.
Radiographs of Fractures and Dislocations. (Twenty plates.)

1904.

FASCICULUS XIII.

PLATES 135, 136, 136^2, 136^3.—Sarcoma Melanodes of Hebra.
Miscellaneous :—
PLATE 137.—Bullous eruption from Flea bites.
,, 138.—Phthyriasis Pubis.
,, 139.—Pityriasis Rosea.
,, 140.—Tinea tonsurans on the chest of an adult.
,, 141.—Tinea tonsurans on the back of an adult.
,, 142.—Lichen Scrophulosorum.

FASCICULUS XIV.

Leucoderma. (Eight plates.)
Myxœdema. (Two plates.)
Miscellaneous.
PLATE K.—Albinism in an Indian.
,, L.—Ossifying cartilaginous tumours of the digits.
,, M.—Unilateral hyperostoses of skull and lower jaw.
,, N.—Rhinophyma.
,, O.—Elephantoid hypertrophy of upper extremity in a child.
Eruptions caused by iodides :—
PLATE 143.—Acute vesicular iodide eruption.
,, 144.—Acute vesicular eruption.

FASCICULUS XV.—Iodide eruptions continued :—

PLATE 145.—Face of woman, Iodide hydroa.
,, 146.—Face of child, Hæmorrhagic iodide eruption.
,, 147.—Face of man, Tuberous iodide eruption.
,, 148.—Face and bust of man, Ulcerating iodide eruption.
,, 149.—Man's hand, Vesicular iodide eruption.
,, 150.—Arm of woman, Iodide hydroa.
,, 151.—Face of woman, Fungating iodide eruption.
,, 152.—Arms of a woman, Iodide (?) hydroa.

FASCICULUS XVI.

PLATES 153-155.—Bromide eruptions.

Lichen urticatus :—
PLATE 156.—Common form in early stage.
,, 157.—Varicelliform and persisting.
,, 158.—Varicelliform.
,, 158 (*bis*).—With secondary impetigo.
,, 159.—Pemphigus Vegetans.

1905.

FASCICULUS XVII.—Fractures and Dislocations of Upper Extremity.

Plates A to K.—Radiographs.

FASCICULI XVIII. and XIX.—Double number.

Plate A.—Elephantiasis in English Subjects. "The Barbadoes Leg."
,, B.—Elephantiasis of lower extremity in Englishmen (three cases).
,, C.—Elephantiasis of lower extremity (two cases).
,, D.—Elephantiasis of lower extremity in a woman.
,, E.—Elephantiasis of Scrotum in two Englishmen.
,, F.—Elephantiasis of Scrotum after Gland disease.
,, G.—Elephantiasis of Scrotum after Gland disease.
,, H.—Lupus Vulgaris on both cheeks.
,, I.—Morphœa Herpetiformis affecting the frontal nerve region.
,, J.—Morphœa Herpetiformis affecting the frontal nerve region.
,, K.—Vaccination Sore on cheek.
,, L.—Fracture of bones of Forearm.
,, M.—Rodent Ulcer on both sides of face.
,, N.—The Potato-like Tumour of Neck (two portraits).
,, O.—The Potato-like Tumour of Neck (portrait and microscopic).
,, P.—Large Tumour in Neck (three views).
,, Q.—Keloid on Scar of burn from Acid.
,, R.—Schistosoma Cattoi (Figs. 1 to 5).
,, S.—Schistosoma Cattoi (Figs. 6 to 9).

1906.

FASCICULI XX. and XXI.—Double Number. Small-pox.

Discrete Small-pox:—
Plates A to E.—A series from the same patient. (An average attack.
Plate A.—5th morning. General distribution.
,, B.—9th ,, ,, ,,
,, C.—11th ,, Hands and arms.
,, D.—12th ,, General distribution.
,, E.—16th ,, Soles of feet.
,, F. Face.
,, G.—Mild Small-pox in the unvaccinated.

Confluent Small-pox:—
Plate H.—9th day.—Pustular eruption on hands and arms.
Plates I. to L.—A series from the same patient (fatal case).
Plate I.—3rd day. Face, early vesicular stage.
,, J.—6th ,, Face, early pustular stage.
,, K.—10th ,, General distribution.
,, L.—13th ,, Face.
Plates M. and N.—Variola Corymbosa.
,, O. to S.—Influence of Vaccination on Small-pox.
Plate T.—Varicella. General distribution.

FASCICULI XXII. and XXIII.—Double Number. Will be devoted to Small-pox and Chicken-pox, with coloured plates.

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